In response to EPA-HQ-OPP-2019-0274-0363

We request an extension to the comment period as 30 days is not enough time to evaluate the risk for all species, including endangered species, in the state of California. This is necessary as the proposed locations in California have not been mentioned. In fact, information on this proposed experiment for California is completely lacking altogether. Therefore, it is necessary for the EPA to provide the necessary information and extend the duration of the comment period so that the public has the ability to properly comment on this issue.

Claims made by Oxitec in documents provided to the FDA(121) and "SECTION G PROPOSED EXPERIMENTAL PROGRAM" along with other data provided to the EPA:

The FDA documents are specifically about OX51A, however, the OX5034 mosquito carries many of the key features of OX513A.

Aedes aegypti males are routinely found in homes. Previous experiments with the release of _Aedes aegypti_ males indicate more larvae will be found indoors than outdoors, and more adult males will be found indoors during such releases. Males are attracted to humans and produce pheromones which attract other males and females. This poses an allergy risk, including inhalant allergies, which has not been evaluated.

According to SECTION G PROPOSED EXPERIMENTAL PROGRAM, 5265 million male mosquitoes are released for the program(EPA). Since the plan is to initially increase the number of *Aedes aegypti* in the test areas, since mosquito derived allergens are present in the air and allergy to insects has a significant bearing on the clinical characteristics of allergic bronchial asthma patients, then these initial releases would likely increase the number or severity of Type I allergic respiratory disorders. There is data for *Aedes aegypti* and inhalant allergens from peer reviewed studies.(133,134,135,168)

With regards to inhalant allergens, documents for this experiment state, *"The circumstances that would allow for the formation and accumulation of airborne particles are not expected to be present at the release sites. Male adult OX5034 will be released into the environment where they seek out females for mating. Ae. aegypti oviposition sites will most likely be present outdoors, in areas that are exposed to rain where water can collect."* However, peer reviewed evidence indicates that *Aedes aegypti* do deposit eggs indoors in homes.(126,127) In fact, peer reviewed evidence, *"found that the infestation of indoor containers by Ae. aegypti was greater than outdoor containers."* and concluded, *"Many studies have demonstrated that Ae. aegypti rest indoors,[38,39] feed indoors[40] and oviposit indoors.[14]."*(Reference 128)

The documents for this experiment also state, *"Further, male adult mosquitoes are the only adult life stage that carry the OX5034 traits, but only adult females seek out the presence of humans as they rely on the blood meal for egg production. Access to indoor spaces is expected to be minimal. However, should access be available, the presence of females will not lead to exposure to the tTAV-OX5034 and DsRed2-OX5034 proteins, as these do not carry the OX5034 traits. While it is possible that males may follow females indoors, it is expected to be a rare event that those males would then remain indoors and*

contribute to dust formation in a significant way." While male adults may be the only adults that carry OX5034 traits, the larvae also express the novel proteins tTAV-OX5034 and DsRed2-OX5034, and it has already been established in the previous paragraph that such larvae will be present indoors in homes. Although this document claims access to indoor spaces is expected to be minimal, this is contradicted by peer reviewed studies which routinely find male *Aedes aegypti* indoors in homes.(171,129,130,141) In fact, in studies where male *Aedes aegypti* were released a greater number of males were often found in homes compared to the number of females in homes and a greater number of mosquitoes, both male and female, were found indoors compared to outdoors.(131) This study used backpack aspirators, whereas in another study comparing BG Sentinel traps to backpack aspirators there was a greater number of *Aedes aegypti* captured in the BG Sentinel traps.(132) Therefore, the number of males in homes is likely greater than the number in the previous study. In the case of the OX5034 releases, where potentially hundreds of millions of *Aedes aegypti* may be released, the number of males will likely significantly increase indoors. It is expected that the release of *Aedes aegypti* males will increase the presence of male *Aedes aegypti* in homes because as more males are present, more males will follow females indoors. Male *Aedes aegypti* also produce pheromones that attract other males and females.(179,180) Therefore, the release of millions of male *Aedes aegypti* will increase the number of males and females around humans. However, even in the absence of females, male *Aedes aegypti* are themselves attracted to humans. *"We used semi-field experiments to demonstrate robust attraction of male Ae. aegypti to humans. Human-baited traps captured up to 25% of released males within 15 min, whereas control traps without humans as bait failed to capture males. Rapid attraction to humans was further demonstrated through videography. Males swarmed around and landed on human subjects, with no activity recorded in paired unbaited controls. The absence of female Ae. aegypti in these experiments rules out a hypothesis by Basrur et al. (2020) that males are attracted not to the human, but to host-seeking females near humans."*(166) Many studies have indicated that male *Aedes aegypti* are attracted to humans and routinely land on humans.(175,176,177,178) Combined with fact that males are attracted to humans, males routinely go indoors and then attract other males and females indoors, the long duration of this experiment of 24 months, and short life expectancy of *Aedes aegypti* males, this is likely to cause male *Aedes aegypti* to die, and hence remain indoors, and contribute to dust formation. If an additional 24 months is added to this experiment, as is proposed, this will only further increase the amount of *Aedes aegypti* males that will enter and die in homes.

It is well established in the peer reviewed literature that *Aedes aegypti* derived allergens, via emanations and detritus, are present in the air and allergy to insects has a significant bearing on the clinical characteristics of allergic bronchial asthma patients. Therefore these initial releases, and increase in overall *Aedes aegypti* numbers, would very likely increase the number or severity of Type I allergic respiratory disorders.(133,134,135,168)

Oxitec failed to provide sequences for transgenic proteins to the public after they were caught failing to identify potential human allergens, including inhalant allergens, in OX513A data provided to the FDA.

Not only may the initial increased number of *Aedes aegypti* lead to increases in allergic responses, but the novel proteins expressed by the OX5034 may also lead to allergic responses. A Report of a Joint

FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology 22 – 25 January 2001 states:

"Cross-reactivity between the expressed protein and a known allergen (as can be found in the protein databases) has to be considered when there is: 1) more than 35 % identity in the amino acid sequence of the expressed protein (i.e. without the leader sequence, if any), using a window of 80 amino acids and a suitable gap penalty (using Clustal-type alignment programs or equivalent alignment programs) or: 2) identity of 6 contiguous amino acids"(6)

There is a risk of the novel proteins expressed by OX5034 being allergens. Sequences for tTAV and DsRed2 specifically from OX5034 must be made available so they can be put into allergen databases. With regards to OX513A, the previous genetically modified mosquito planned to be released, Oxitec claimed to the FDA that there were no allergens found in allergen databases that matched the sequences for tTAV and DsRed2 from OX513A. However, when we put these sequences into the Structural Database of Allergenic Proteins, using FAO/WHO Allergenicity Rules based on Sequence Homology and exact match for 6 contiguous amino acids, several sequence matches were identified which correspond to allergen sequence matches including inhalant allergens. Therefore, the experimenter, Oxitec, should be transparent and make these sequences publicly available so that we can identify allergens that Oxitec may have failed to identify since this has already occurred with the OX513A.

When performing a search for OX513A through SDAP - Structural Database of Allergenic Proteins, and using FAO/WHO Allergenicity Rules based on Sequence Homology, and exact match for 6 contiguous amino acids, several matches were identified(122).

For the predicted amino acid sequence of tTAV given in Figure 18 in the FDA document(121), the following sequence matches were identified which corresponds to the following allergen :

1. "LRQAIE" = Allergen Ric c 1, Sequence: P01089

For the Amino Acid Sequence of the DsRed2 protein found in Figure 19 in the FDA document(121), the following sequence matches were identified which corresponds to the following allergens :

1. "SFPEGF" = Allergen Bet v 1.at87, Sequence: CAA07328

2. "SFPEGF" = Allergen Bet v 1.at14, Sequence: CAA07320

3. "SFPEGF" = Allergen Bet v 1, Sequence: P43183

4. "SFPEGF" = Allergen Bet v 1.0601, Sequence: P43179

5. "SFPEGF" = Allergen Bet v 1.0101, Sequence: P15494

6. "SFPEGF" = Allergen Bet v 1.2501, Sequence: CAB02156

7. "SFPEGF" = Allergen Bet v 1.0101, Sequence: CAA33887

8. "SFPEGF" = Allergen Bet v 1.at8, Sequence: CAA07318

9. "SFPEGF" = Allergen Bet v 1.2601, Sequence: CAB02157

10. "SFPEGF" = Allergen Bet v 1.0601, Sequence: CAA54484

11. "SFPEGF" = Allergen Bet v 1.0801, Sequence: CAA54487

12. "SFPEGF" = Allergen Bet v 1.2401, Sequence: CAB02155

13. "SFPEGF" = Allergen Bet v 1.at5, Sequence: CAA07329

14. "SFPEGF" = Allergen Bet v 1.at50, Sequence: CAA07326

15. "SFPEGF" = Allergen Bet v 1, Sequence: CAA05188

16. "SFPEGF" = Allergen Bet v 1, Sequence: CAA05189

17. "SFPEGF" = Allergen Bet v 1.3001, Sequence: CAB02161

18. "SFPEGF" = Allergen Bet v 1.1501, Sequence: Q42499

19. "SFPEGF" = Allergen Bet v 1.at42, Sequence: CAA07324

20. "SFPEGF" = Allergen Bet v 1.2301, Sequence: CAA96545

21. "SFPEGF" = Allergen Bet v 1.at37, Sequence: CAA07323

22. "SFPEGF" = Allergen Bet v 1.2201, Sequence: CAA96547

23. "SFPEGF" = Allergen Bet v 1.2901, Sequence: CAB02160

24. "SFPEGF" = Allergen Bet v 1.1501, Sequence: CAA96538

1. "DGGVAT" = Allergen Sola t 1, Sequence: P15476

2. "DGGVAT" = Allergen Sola t 1, Sequence: AAA33819

1. "GGVATV" = Allergen Sola t 1, Sequence: P15476

2. "GGVATV" = Allergen Sola t 1, Sequence: AAA33819

1. "ERTEGR" = Allergen Api c 1.0101, Sequence: AF321087

2. "ERTEGR" = Allergen Api m 1, Sequence: P00630

Sequence matches were identified which correspond to human allergen sequence matches, including inhalant allergens, from castor beans, eastern honey bee, white birch tree and potatoes. Castor bean inhalant allergens may result in anaphylactic shock(136), and the inhalant Bet v 1 allergen from white birch, which shares sequences with the OX513A, affects over 100 million allergic patients.(137) While sharing matching sequences alone doesn't guarantee an allergic reaction, further studies are required when there are exact matches for 6 contiguous amino acids, and it appears no studies have been done. When Oxitec submitted documentation to the EPA for OX5034, however, they did not publicly provide the sequences for either protein making it impossible for physicians and scientists to search the allergen databases.

Dermal exposure will be frequent because male *Aedes aegypti* are attracted to humans so frequently land on humans and they produce pheromones that attract other males and females. Females carrying sperm from OX5034 males will also land on humans.

Dermal exposure to OX5034 may also play a role in increased allergies. The EPA states, *"The only other potential route of dermal exposure to these proteins would be if a male OX5034 mosquito should alight on the bare skin of a human, and the human crushed the mosquito onto the bare skin. However, given that males do not feed on humans, the frequency of human interaction with male mosquitoes is expected to be minimal."* Many studies have indicated that male *Aedes aegypti* are attracted to humans and routinely land on humans.(166,175,176,177,178) Male *Aedes aegypti* also produce pheromones that attract other males and females.(179,180) Therefore, the release of male *Aedes aegypti* will increase the number of males and females around humans. However, this is not the only potential dermal route, as *Aedes aegypti* females often mate and store male sperm in the spermatheca for life.(157) Therefore, if these novel proteins are present in the OX5034 sperm, every female that mates with a male OX5034 will be carrying these proteins and females continuously interact with humans throughout their life. The question of whether or not these novel proteins, or any alteration in proteins that may increase allergies, are present in the seminal fluids of OX5034 remains unanswered.

Female OX5034 may be released and they may have an increase in the levels of allergens in their saliva compared to wild *Aedes aegypti* in the test areas.

According to the mutation rates some OX5034 females may be released and some people will be bitten.(125) Since there are at least 8 allergens that have been found in *Aedes aegypti* saliva(81), an initial increase in the number of *Aedes aegypti* females could increase the number of allergic reactions in the Keys and California The reactions could include large local swellings and redness, generalized urticaria, angioedema, nausea, dizziness, headaches, lethargy and systemic anaphylaxis.(82) Oxitec also does not seem to indicate if there are differences in the levels of proteins in the saliva of the GE mosquitoes compared to wild mosquitoes in the Florida Keys or California. Since there are at least 8 allergens that have been found in *Aedes aegypti* saliva(81), an increase in these levels of allergens in GE mosquitoes may increase allergic responses or increase severity of allergic responses in people in the test area bitten by these GE female *Aedes aegypti*. Oxitec must therefore also conduct studies to

determine if there are differences in the allergen levels of their GE *Aedes aegypti* compared to wild *Aedes aegypti* currently found in the Keys and California.

Wild females that mate with OX5034 may have increased levels of allergens in their saliva, or novel allergens compared to wild females that mate with wild males.

Oxitec must also conduct studies to determine if the saliva of wild female *Aedes aegypti* in the Keys and California are altered once they are inseminated by OX5034 *Aedes aegypti* males as it is unknown if this may alter the saliva of the mated wild female and perhaps even cause the mated wild female to have proteins unique to OX5034 x wild *Aedes aegypti* in their saliva.

Also, allergen databases are often incomplete and therefore the risk of an allergic response in residents exposed to the GE mosquitoes is a possibility and residents must be informed of and consent to such a risk. If all residents in the test area do consent Oxitec must provide a physician, as a part of the test, who will monitor the health of the residents that are exposed to Oxitec's mosquitoes. In the case of an adverse event being reported during this trial Oxitec must have a plan in place to recall the mosquitoes and/or evacuate the residents. This would involve erecting temporary structures outside of the test area, in case of an adverse event being reported, to evacuate residents to. An immediate response plan to eradicate the mosquitoes must also be in place since the lethality trait cannot be fully relied on considering 50%, all male, offspring can survive and an even greater percent when they exposed to pet food, a likely scenario, or environmental tetracyclines.(112)

Even in the absence of tetracyclines Oxitec's mosquitoes are likely to remain in the environment due to the offspring that survive to adulthood without exposure to tetracycline. The Food and Agriculture Organization of the United Nations agrees on their website stating, *"The transgenic approaches instead can have potentially unforeseen consequences because the released insects are not sterile and therefore will reproduce and become established."*(91)

Oxitec was asked whether health studies were conducted on humans who were bitten by GE mosquitoes, they replied that many of the scientists working with the GE mosquitoes had been bitten and no adverse health effects were reported.(9) So, all they have is anecdotal evidence from their own staff. This is insufficient when human health is potentially at risk.

The receipt of seminal fluid proteins that are transferred from males to females along with sperm during copulation may cause changes in host-seeking and feeding behavior(94). Therefore, Oxitec must conduct studies to determine if there are differences in seminal fluid proteins and sperm of their GE mosquitoes compared to wild male mosquitoes in the Florida Keys and California and if any differences are observed what impact this has on the host-seeking and feeding behavior of wild *Aedes aegypti* females that mate with Oxitec's GE male mosquitoes. This is especially important since an increase in multiple host feeding could increase allergy risk and multiple host feeding may affect the spread of diseases mosquitoes carry. (31) Once Oxitec has published these necessary studies and they are replicated by independent experts, then Oxitec must reassess and include this new information. Until Oxitec has conducted such studies and they are replicated by independent experts their GE mosquitoes cannot be considered safe.

Transgenic proteins expressed by OX5034 may be allergens and may be present in saliva of females that may be released. These potentially allergenic novel proteins expressed by males may be inhalant allergens. Testing is necessary.

If the earlier estimates on mutations are correct, some GE female mosquitoes will be present at some point in the Keys or California(125) What happens if people are bitten? While Oxitec previously claimed there are no proteins unique to the GE mosquito in the saliva of the OX513A mosquito, no data is presented for OX5034. Even if Oxitec provided data for a few hundred or thousand OX5034, it is still possible that some percent of OX5034 mosquitoes do have these proteins in their saliva. Therefore, toxicity and allergenicity studies must be conducted to determine what happens if people are bitten by OX5034 with the transgenic proteins expressed in their saliva. Such data, if it exists, does not appear to have been published in a peer reviewed journal, or replicated by independent experts. Since reproducibility is one of the main principles of the scientific method, these studies must be reproduced by independent experts. This can be done through programs such as PLOS ONE's Reproducibility Initiative.(76)

It has already been established that proteins from the OX513A share sequences identical to known human allergens and this is likely true for OX5034 as well. These proteins could be present in the saliva of OX5034 females or as inhalant allergens from OX5034 males. According to A Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology , *"Since identity of 6 contiguous amino acids has an appreciable risk of occurring by chance, verification of potential crossreactivity is warranted when criterion (1) is negative, but criterion (2) is positive. In this situation suitable antibodies (from human or animal source) have to be tested to substantiate the potential for crossreactivity"*(6) Therefore, Oxitec must perform such tests to assess allergenicity. Since reproducibility is one of the main principles of the scientific method, these studies must be reproduced by independent experts. This can be done through programs such as PLOS ONE's Reproducibility Initiative.(76) Once Oxitec has conducted these necessary studies and they are replicated by independent experts, then Oxitec must reassess and include this new information. Until Oxitec has conducted such studies and they are replicated by independent experts their GE mosquitoes cannot be considered safe.

Male, and potentially female, OX5034 released may increase risk of Zika and other mosquito borne diseases. Venereal transmission, Transovarial transmission and Male accessory gland proteins, which play a significant role in disease transmission, have not been evaluated.

According to the mutation rates some OX5034 females may be released and some people will be bitten.(125) Even if no females are released the OX5034 mosquitoes may still increase mosquito-borne diseases. If the OX5034 mates with wild females harboring these diseases the male can then acquire diseases such as Zika from the infected female through venereal transmission.(139,140,141) Since male *Aedes aegypti* can mate as many as 6 times per day(142), releasing hundreds of millions of males is a

really bad idea as this could cause a rapid spread of a disease like ZIKV. Zika was previously present in the Florida Keys making this a serious concern.(143)

A recent study indicates that a different genetically modified mosquito was better able to transmit malaria.(138) Which means that some genetic modifications to mosquitoes could cause greater transmission of mosquito-borne diseases, thus increasing the number of cases of human diseases such as dengue fever, Zika virus or chikungunya, or even animal diseases such as canine and feline heartworm or avian malaria that could impact the endangered or threatened Southern bald eagle, Roseate tern, Everglade snail kite, Cape Sable seaside sparrow, Bachman's warbler, Wood stork, Piping Plover and Red knot in Monroe county, Florida. Therefore, Oxitec cannot rely on vectorial capacity for only the host species OX5034 before transformation, and disease transmission testing, including transovarial transmission and venereal transmission testing, must be done on the transformed OX5034 and the offspring of transformed OX5034 x wild *Aedes aegypti* present in the Florida Keys and California to determine the vector competence.

Peer reviewed studies routinely find male *Aedes aegypti* indoors in homes.(171,129,130,141) In fact, in studies where male *Aedes aegypti* were released a greater number of males were often found in homes compared to the number of females in homes and a greater number of mosquitoes, both male and female, were found indoors compared to outdoors.(131) Many studies have indicated that male *Aedes aegypti* are attracted to humans and routinely land on humans.(166,175,176,177,178) Male *Aedes aegypti* also produce pheromones that attract other males and females.(179,180) Therefore, the release of male *Aedes aegypti* will increase the number of males and females around humans. This increase in females around humans may cause an increase in mosquito-borne diseases.

Other strategies to control the *Aedes aegypti* also rely on releasing male mosquitoes, however, strategies such as the use of the bacterium *Wolbachia* do not carry this risk of spreading diseases as *Wolbachia* inhibits viral replication in infected *Aedes aegypti* stopping the mosquito from spreading diseases such as Zika virus, dengue fever,yellow fever, chikungunya as well as the parasites that cause avian malaria and the filarial nematodes responsible for canine and feline heartworm.(144,145) Unlike OX5034, Wolbachia-infected mosquitoes could reduce the *Aedes aegypti* population without the risk of increasing mosquito-borne diseases.(146) In fact, a study done in Australia where *Wolbachia* infected mosquitoes were released show a 96% reduction in dengue fever.(147)

On the other hand, a study in Brazil using a previous version of a genetically modified mosquito, OX513A, found that cities that did not use these genetically modified mosquitoes had lower rates of dengue in 2017, compared to 2015 rates, than cities that did use the OX513A.(148) The situation with OX5034, however, could be far worse than with OX513A because, unlike the OX5034, the OX513A were not supposed to be able to produce any offspring that survived to adulthood. Although the claim that OX513A could not produce any offspring that survived to adults was false and indeed 3-4%, or as much as 15% or higher of the offspring exposed to cat food, did survive to adulthood(149), this is a far smaller number than around 50% of offspring, the males, surviving to adulthood for OX5034. Which brings up another problem, if male OX5034 mate with wild females harboring Zika virus, for example, not only can the male acquire Zika from the female through venereal transmission, but the offspring of this pair can acquire these diseases through vertical transmission.(150) That means that not only can the OX5034 that are released spread Zika, but their male, and likely some female, offspring can spread Zika as well. Now these male offspring can harbor diseases like dengue fever(56) and chikungunya, for example, and they could infect wild females through venereal transmission spreading these mosquito-borne diseases even

more.(27,28) It is even possible that OX5034 has higher rates of venereal transmission for mosquito-borne diseases compared to wild *Aedes aegypti* in the Florida Keys, or higher rates of vertical transmission to the offspring of OX5034, but there is no way to know since there was no data in the EPA documents and no peer reviewed studies examining this issue. In fact, there appears to be no peer reviewed studies examining any issues with OX5034.

Yet another way OX5034 mosquitoes could increase mosquito-borne diseases is if there are differences in the seminal fluids of the male genetically modified mosquitoes. When a female *Aedes aegypti* receives seminal fluid and sperm from a male this causes physiological responses in the female. These physiological responses could result in changes in host seeking behavior, blood meal size, blood digestion rate, life expectancy, re-mating behavior, egg development or fecundity.(151) Mated female *Aedes aegypti* also may be more attracted to human odors(175) Any, or all, of these changes could increase mosquito-borne disease transmission. If a female mosquito lives longer, for example, she may be able to have more offspring spreading the diseases further, or if she has a change in host seeking, such as biting more people than usual, she could spread diseases more. Since seminal fluids can have such a profound effect on female mosquitoes and disease transmission, proteome testing of the OX5034 seminal fluid is necessary to see if there are differences compared to wild *Aedes aegypti*'s seminal fluids in the Florida Keys and California.

Even in the absence of the transgenes, the OX5034 is still a foreign strain of *Aedes aegypti* which is not currently present in the Florida Keys or California. According to Oxitec, for the OX5034 a laboratory strain was mixed with a Latin American wild strain which has a background comprising genetics from ten different colonies of *Aedes aegypti* originating in Mexico. This has led to concerns that this hodge podge, lab strain mixed with Mexican strain mixed with the strain present in the Florida Keys, California or elsewhere, could lead to hybrid vigor. Hybrid vigor can lead to a more robust population of mosquito than those which are not a mixture of different strains.(152)

As the EPA documents do not contain any mention of venereal transmission, transovarial transmission, or seminal fluids. No data was presented by Oxitec for sexual transmission or vertical transmission of mosquito-borne diseases, or testing of male accessory gland proteins despite the fact that these all play an important role in the transmission of vector-borne diseases. Therefore, this data must be presented and evaluated.

Changes in saliva of Female OX5034 or wild females that mate with male OX5034 could increase disease transmission.

Aedes aegypti saliva is composed of numerous proteins which can play a role in disease transmission.(184) For example, the protein, "aegyptin," has been found in decreased abundance in the saliva of *Aedes aegypti* infected with dengue.(183) Changes in the levels of proteins in saliva of female OX5034 due to the transformation process, or changes in the saliva of wild females that mate with OX5034 males could cause an increase in disease transmission. Therefore, the saliva of *Aedes aegypti* females must be tested and compared to wild *Aedes aegypti* saliva in the Florida Keys and California. The saliva of wild *Aedes aegypti* females in the Florida Keys and California that mate with OX5034 males must

also be tested and compared to wild *Aedes aegypti* females that mate with wild males in the Florida Keys and California.

Mated females, which live longer and take larger blood meals, will increase with more males released and may increase spread of diseases. A lower population of females could also increase spread of diseases through an increase in multiple host feeding.

A reduction in the *Aedes aegypti* population may not necessarily mean a reduction in dengue fever(29). Studies suggest *Aedes aegypti* are opportunistic feeders (30); if there is less competition, the remaining mosquitoes may feed on more people than usual. Multiple host feeding may affect the spread of diseases mosquitoes carry.(31) This is especially important since the release of millions of additional males will likely increase the number of mated females and mated females live longer, take larger blood meals and may be more attracted to human odors which could increase the spread of mosquito-borne diseases.(151,175) Under normal conditions there are likely females in the wild that die virgins. Although some males are very good breeders and have mastered harmonic convergence, other males are not. Also the distance needed to find a female in the environment makes mating success much less than 100%. Even in laboratory experiments under optimal conditions female insemination rates often do not reach 100% and those rates are significantly lower in large low-density field cages.(153) This would indicate that females are even more likely to fail to find a mate in the open environment. However, by substantially increasing the number of males, as takes place during this experiment, the number of virgin females will initially decrease as more males will be available for breeding. Virgin females obviously do not pass on diseases to their mates or their offspring so there is less risk of disease spread. Mathematically, if there are only a few Zika infected females in the wild in Florida or California, the smaller the number of males in the wild the less likely one of those males will find one of those infected females and mate. As large numbers of additional males are released it dramatically increases the odds of some of these males finding these infected females and becoming infected via venereal transmission. Now these infected males potentially go out and mate with other females spreading Zika. These females will then bite hosts, spreading Zika to humans. This will allow other wild females to acquire Zika from those infected humans via bite as well, and have male offspring that acquire Zika via transovarial transmission and then spread it via venereal transmission. Wild females that mate with wild males will also be able to bite these infected humans and have male and female offspring able to spread Zika. Now as the mosquitoes start to decrease in population, provided this experiment works as intended, there will be more Zika infected females than at the start.

Another problem is that as male population density increases this increases the likelihood of incomplete sperm transfer as other males will be interrupting the mating to try to mate themselves. When this happens, females tend to mate twice or more.(153,154) The more a female mates the more disease spreads as well. So adding all of these extra males also increases the risk of more mated females and more females having multiple mates.

An increase in multiple host feeding could increase allergy risk and multiple host feeding may affect the spread of diseases mosquitoes carry.(31) The receipt of seminal fluid proteins that are transferred from males to females along with sperm during copulation may cause changes in host-seeking and feeding

behavior(94). Therefore, Oxitec must also conduct studies to determine if there are differences in seminal fluid proteins and sperm of their GE mosquitoes compared to wild male mosquitoes in the Florida Keys and if any differences are observed what impact this has on the host-seeking and feeding behavior of wild *Aedes aegypti* females that mate with OX5034 male mosquitoes.

As the OX5034 releases could alter dengue transmission, it should be noted that complex immune responses to the four types of dengue virus mean that a partial reduction in mosquito numbers can reduce cross-immunity to the different serotypes and increase the number of cases of the severe form of the disease, dengue haemorrhagic fever, which is more likely to be fatal. Success in reducing illness in young children can also mean more delayed and serious cases of dengue.(45)

Since the release of millions of additional males will likely increase the number of mated females and mated females live longer, take larger blood meals and may be more attracted to human odors which could increase the spread of mosquito-borne diseases(151,175) and these mated females require human blood to reproduce and have the offspring of the OX5034, a purpose of this experiment, this must be considered a human experiment. Since humans are directly involved and are required for this experiment, this experiment must abide by all requirements for an experiment on humans including, but not limited to, informed consent.

Aedes albopictus may move in as _Aedes aegypti_ population decreases causing a co-occurence of both species creating a scenario where two vectors of diseases will be present in areas where only one was present before the trial. This co-occurrence of two species would also cause an increase in the number and severity of allergic reactions as people in test areas would then be exposed to an additional species that has allergens which they are not desensitized to.

Current control methods in Florida, such as the use of the larvicide Vectobac - Bacillus thuringiensis israelensis, often target both *Aedes aegypti* and *Aedes albopictus*.(85,86,) However, the OX5034 mosquitoes would target only *Aedes aegypti* possibly causing a reduction in only the *Aedes aegypti* population which could create an opportunity for *Aedes albopictus* to enter an area and establish itself. This could create a situation where *Aedes aegypti* and *Aedes albopictus* co-occur. The *Aedes albopictus* also transmits mosquito borne diseases such as dengue fever and chikungunya(44) as well as Zika(90). *Aedes albopictus* infected with dengue, as well as West Nile Virus, eastern equine encephalomyelitis, Cache Valley and La Crosse virus have been found in North America.(161,162,163,164)

Aedes albopictus has replaced the *Aedes aegypti* in some urban environments in the past such as in Mobile, Alabama.(32) Therefore, if the *Aedes aegypti* population is reduced it may create an environment where the *Aedes albopictus* would thrive in areas where only *Aedes aegypti* currently exists. This has been considered a likely scenario for the GE mosquito releases in Panama.(65) *Aedes albopictus* are already found in some parts of the Florida Keys(53) and California(182), making it likely that *Aedes albopictus* will move into the test areas in the Keys or California where *Aedes albopictus* is not currently present. Once the trial is over and *Aedes aegypti* population increases again this could create a situation where *Aedes aegypti* and *Aedes albopictus* co-occur. Since areas in the Keys and California are

suburban areas and *Aedes aegypti* and *Aedes albopictus* already co-occur in other suburban areas of South Florida(83) and California(182) this is a likely scenario for the test areas in the Keys and California as well. So by reducing only the *Aedes aegypti* population in parts of the Keys or California where *Aedes albopictus* is not present it can create a scenario where there are now 2 different species which spread the same diseases instead of just 1. This is extremely relevant since, for example, data strongly suggests that *Aedes albopictus* acted as the major vector of both dengue and chikungunya in Libreville in 2007, impacting on the epidemiology of both viruses in this area, even though both *Aedes aegypti* and *Aedes albopictus* were present.(44) Therefore, outbreaks can occur which are caused by *Aedes albopictus* which do not involve *Aedes aegypti* and vice versa. So this release could create a scenario where 2 separate species can independently cause an outbreak of such diseases instead of only 1 species which currently exists. The EPA responded to comments about *Aedes albopictus* displacing the *Aedes aegypti* in the test areas, but the EPA did not respond to the concerns about co-occurrence of *Aedes aegypti* and *Aedes albopictus* in the test area because of the experiment with OX5034.

Although at least 8 allergens have been found in *Aedes aegypti* saliva, more than 16 allergens have been found in *Aedes albopictus* saliva.(81) If *Aedes albopictus* are able to establish in the trial area because of a reduction in *Aedes aegypti* this could increase the number of allergic reactions. Since most people are bitten by mosquitoes in or around their home, Keys and California residents are not likely to have been largely exposed to *Aedes albopictus* and therefore natural desensitization likely does not exist among Keys and California residents in the test areas. Therefore, due to Keys and California residents low or absent natural immunity, having little or no previous exposure to *Aedes albopictus*, they are at an increased risk of severe reactions to mosquito bites and this is especially true for young children.(84) OX5034 mosquitoes present a unique risk since current control methods in the Keys and California, such as the use of the larvicide Vectobac - Bacillus thuringiensis israelensis(86), often target both Aedes aegypti and *Aedes albopictus*(85) However, OX5034 mosquitoes would target only *Aedes aegypti* possibly causing a reduction in only *Aedes aegypti* which could create an opportunity for *Aedes albopictus* to enter the Keys and California and establish itself due to reduced competition with *Aedes aegypti*, whereas current control methods do not carry the same risk.

Since as much as 18.4% of OX513A offspring could survive when exposed to pet food, which they are exposed to in the Florida Keys and California, it is extremely unlikely that the OX5034 female mosquitoes will all die, and extremely likely that the Oxitec mosquitoes will survive beyond the duration of the trial. Oxitec must reassess and include this new information. Even in the absence of tetracycline Oxitec's mosquitoes are likely to remain in the environment due to the offspring that survive to adulthood without exposure to tetracycline. The Food and Agriculture Organization of the United Nations agrees on their website stating, *"The transgenic approaches instead can have potentially unforeseen consequences because the released insects are not sterile and therefore will reproduce and become established."*(91) This makes it extremely unlikely that all *Aedes aegypti* will be eliminated from the trial area and if *Aedes albopictus* moves in these two species will co-occur.

Tetracycline use can increase the risk of tetracycline resistant bacteria, may enhance susceptibility of male, and potentially female, OX5034 to mosquito-borne diseases, and waste water from breeding OX5034 could contribute to antibiotic resistant bacteria even if not disposed of in the U.S.

Researchers concluded that although, *"Moll et al. (2001) describe an effective mechanism to eliminate gut microorganisms during mosquito metamorphosis and adult emergence. Bacteria found in recently emerged non-fed adults are present from the larval and pupal stage and, therefore, have some adaptations to overcome this mechanism."*(98) Other researchers have concluded that based on their observations Serratia odorifera was transstadially transmitted in *Aedes aegypti* from larvae to adult, and that Serratia odorifera enhances its susceptibility to dengue-2 virus.(99) Other studies provide similar results(107). A recent study suggests that *Aedes aegypti* larvae cannot survive past the first instar without gut microbiota. In this study they used ampicillin to kill the gut bacteria in second and third instar *Aedes aegypti* and they did not molt. However, if the *Aedes aegypti* larvae had ampicillin resistant bacteria they survived to adulthood.(109) If we assume this research is correct and microbiota are needed for *Aedes aegypti* to survive, we can also assume OX5034 mosquitoes are like conventional mosquitoes and also require microbiota to survive. Since OX5034 female mosquitoes are exposed to tetracyclines this would likely kill all of the microbiota except the tetracycline resistant bacteria. Therefore, there is a causal pathway for genetically engineered *Aedes aegypti*'s gut bacteria acquiring antibiotic resistance genes as they are fed on antibiotics in the laboratory. Some evidence suggests a correlation between tetracycline resistance in the Cayman Islands and Oxitec's GE mosquito release there.(110) A postgraduate student working with Oxitec's GE *Aedes aegypti* mosquitoes has conducted relevant experiments which found that *"Colonies grew on plates supplemented with 50 μg ml-1 of chlortetracycline, indicating that the larvae bore chlortetracycline resistant bacteria"*.(119) Therefore, Oxitec must conduct studies to determine if tetracycline resistant microbiota are found in GE mosquitoes.

There is some evidence that antibiotics may increase the transmission of dengue fever by *Aedes aegypti* mosquitoes.(120) The EPA replied to the potential of the exposure to antibiotics to increase vector competence stating, *"EPA carefully considered the possibility that treatment with antibiotics during colony production could affect vector competency. However, EPA does not find that this consideration affects its analysis because (1) only OX5034 males will form part of the testing"*. This, however, does not take into consideration that even if no females are released the OX5034 mosquitoes may still increase mosquito-borne diseases. If the OX5034 mates with wild females harboring these diseases the male can then acquire diseases such as Zika from the infected female through venereal transmission.(139,140,141) Since male *Aedes aegypti* can mate as many as 6 times per day(142), releasing hundreds of millions of males is a really bad idea as this could cause a rapid spread of a disease like ZIKV. Zika was previously present in the Florida Keys making this a serious concern.(143)Therefore, testing must be done to determine if male OX5034 have higher vector competence via venereal transmission compared to wild male *Aedes aegypti* in the Florida Keys and California.

Tetracycline resistant bacteria could also be found on GE *Aedes aegypti* due to the potential for tetracycline resistant bacteria in the laboratory where tetracycline is used and GE *Aedes aegypti* are potentially exposed. Therefore, Oxitec must conduct studies to determine if tetracycline resistant bacteria are found on GE *Aedes aegypti* as well.

In order to breed GE mosquitoes Oxitec must use the antibiotic tetracycline in the process. The waste from this tetracycline has the potential to increase antibiotic resistant pathogens.(64) Although the EPA responded, *"Similarly, because no tetracyclines will be used in the US facilities producing OX5034 male adults for release in the United States, nor will tetracyclines be used in the release devices for field*

deployment of OX5034 mosquito eggs, the question of whether disposal of wastewater could spread antibiotic resistance does not apply." This does not take into consideration that the waste will be discarded somewhere, whether in the U.S. or not, and this waste has the potential to increase antibiotic resistant pathogens.(64)

Tetracycline is an antibiotic used for humans and may therefore cause human harm if pathogens become resistant to it.(110) This antibiotic is used to treat MRSA and its non-medical use may lead to tetracycline resistant MRSA.(123) Tetracycline is also used in the treatment of canine heartworm which is transmitted by *Aedes aegypti*.(124) Therefore, tetracycline resistant bacteria harbored on Oxitec's mosquitoes could render tetracycline useless for canine heartworm treatment.

Aedes aegypti can escape the trial area as they can fly 2,500 meters or more, routinely travel by vehicle, are routinely found indoors and can survive cold weather, survive to adulthood in water with high salinity and have eggs that can resist desiccation. Oxitec's own study suggests genetically modified mosquitoes may travel as much as 1,490 meters.

Although Oxitec claims, *"Geographic containment is provided by the siting of the egg production unit in the UK, which is beyond the isothermal range of the mosquito (i.e., it is too cold for Ae.aegypti to survive outside the climate controlled environment of the laboratory)"*(121), *Aedes aegypti* have been found in the UK outside of Oxitec's laboratory.(159) *Aedes aegypti* also routinely deposit eggs indoors in homes where temperature is controlled.(126,127) In fact, peer reviewed evidence, *"found that the infestation of indoor containers by Ae. aegypti was greater than outdoor containers."* and concluded, *"Many studies have demonstrated that Ae. aegypti rest indoors,[38,39] feed indoors[40] and oviposit indoors.[14]."*(Reference 128) Recent evidence suggests these mosquitoes may survive outside of a lab even in colder climates and *Aedes aegypti* eggs can remain viable after exposure to subzero temperatures.(73,167) Peer reviewed evidence even suggests a population of *Aedes aegypti* in a cold climate were originally from Florida.(174) Oxitec must reassess and include this new information.

Although Oxitec claims that, *"Geophysical containment is provided by the island location of the release site, where the site is predominantly surrounded by ocean, and the mosquito in any life stage cannot survive due to the high salinity of the waters."*(121) Islands in the Florida Keys are not isolated island locations and California is not an island. The Keys are connected via roadway to the state of Florida, which is then connected via roadway to the rest of the continental U.S. So for the question of *"Will the genetically engineered(GE) mosquitoes travel outside of the Keys?"* The answer is an almost definite *"Yes"*. Some studies observed *Aedes aegypti* traveling up to 800 meters(59) as much as 1000 meters across water and up to 2,500 meters in some cases(96). In the 1960's the U.S. military demonstrated that when coupled with ocean winds *Aedes aegypti* could travel up to 3 1/2 miles to shore(106). This distance could easily place a genetically engineered mosquito in a vehicle intended for another state or another country. Mosquitoes are believed to frequently travel long distances via boat, automobile, etc.(100) and this is also likely true for Florida.(101). It is believed that the recent presence of *Aedes aegypti* in California was caused by commerce via air, railroad, or trucks traveling from the southern U.S.(102). Even *Aedes aegypti* found as far away as the Netherlands are believed to have traveled there in airplane tires arriving from southern Florida.(103,104) With over 3 million visitors to the Florida

Keys(25) a year, and numerous vehicles traveling in and out of the area, these genetically engineered mosquitoes escaping the test area is an extremely likely scenario. However, despite Oxitec's misinformation, *Aedes aegypti* are fully capable of breeding in water with high levels of salinity.(105,169) *Aedes aegypti* eggs may stay viable and resistant to desiccation for up to 450 days(34). If OX5034 mosquitoes, or the female they mate with, escape the test area there is a good likelihood they would successfully mate and/or deposit eggs in other areas.

Although Oxitec claims in SECTION G PROPOSED EXPERIMENTAL PROGRAM, *"Distance between TA's and UCA's greater than 400m (as recommended by the World Health Organization)"*, *"Treated areas will be located greater than 400m from commercial citrus growing areas"* and *"To monitor dispersal a network of BG Sentinel traps (minimum of 30) will be spread out to a maximum distance of 400m a distance between the Treatment Area (TA) and the Untreated Comparator Area (UCA) of 400m"*, or 500m as was permitted by the EPA, this is inadequate. Since some studies observed *Aedes aegypti* traveling 800 meters(59) as much as 1000 meters across water, up to 2,500 meters in some cases(96) and when coupled with ocean winds *Aedes aegypti* could travel up to 3 1/2 miles to shore(106), this distance is not adequate to stop potential immigration of other *Aedes aegypti* into the treatment area or Oxitec's mosquitoes from immigrating into the untreated comparator area. This does not even include the fact that the Oxitec mosquitoes or other mosquitoes could still enter a vehicle and travel via vehicle from one area to another. This is especially important since the increase in males will increase mating competition and potentially drive already existing *Aedes aegypti* from the treatment area into the untreated comparator area which would skew the results in favor of reduction in the treatment area. Since Oxitec states, *"their dispersal by spontaneous flight is less than 200 m"* it would seem they are unaware of the observations that *Aedes aegypti* can travel more than 800 meters(59,96) and therefore they must reassess based on this new information and increase the buffer zone and trap placement to at least greater than 2,500 meters(96). The EPA has replied to this stating, *"Although longer dispersal distances for Ae. aegypti have been observed, a compilation of release recapture studies around the world found that most Ae. aegypti are recovered within 20 m to 50 m of the release point, with a small percentage found 170 m but generally not more than 200 m from the release point12."* The reference for this comes from an OECD document that states, *"Experiments in different parts of the world involving the release and recapture of adults suggest that most are recovered within 20 m to 50 m of the release point, with a small percentage reaching distances greater than 170 m and not more than 200 m (Morlan and Hayes, 1958; Sheppard et al., 1969; McDonald, 1977; Trpis and Häusermann, 1986; Rodhain and Rosen, 1997; Muir and Kay, 1998; Ordoñez-Gonzalez et al., 2001; Harrington et al., 2005; Russell et al., 2005; Maciel-de-Freitas, Codeço and Lourenço-de-Oliveira, 2007a, 2007b; Valerio et al., 2012)."* The references cited by the OECD do not include the references provided here which show distances much greater than 200 meters(59,96). In fact, Oxitec's own study concluded that OX513A had a flight potential of 1490 meters.(156)

The EPA argues that, *"Should mosquitoes be transported or otherwise dispersed beyond the test area, OX5034 is not expected to establish in areas outside of the test area for the same reason it is not expected to establish within the test area as discussed in EPA's response to Unit VI.A. This is because the OX5034 trait is self-limiting and thus is expected to be eliminated from the Ae. aegypti population regardless of whether that population is within or outside of the test area."* This ignores that the OX5034 trait is self-limiting only in the absence of tetracyclines. If the OX5034 mosquitoes are dispersed to an area where tetracyclines are used these OX5034 will not only remain in the environment, but females

will also be released. As the EPA has not evaluated the allergenicity or toxicity of saliva from OX5034 females this is a risk that has not, and must be evaluated by the EPA.

"There are a variety of ethical issues that are raised from the use of genetically modified insects" wrote Darryl Macer, who has published papers on the subject for the World Health Organization and in academic journals. *"But the most challenging may be the process of informed consent for individuals and communities. Each community or society needs to be given a chance to set consensus."*(35)

Since these mosquitoes will likely travel by vehicle to locations outside of the Florida Keys, the lack of informed consent by communities outside of the Keys and California creates ethical issues.(112) This is especially important since a survey suggests more informed people disagree with the release of genetically engineered mosquitoes than agree, and over 237,000 people signed a petition opposing the release of these mosquitoes.(54,55) Oxitec says for mosquito larvae that develop to adulthood, the GE mosquito would still be contained because they only travel a hundred yards or so in their lifetime.(9) However, that doesn't take into account that the Keys are a tourist area visited by an estimated 3 million people from various parts of the U.S. and other countries every year(25) and mosquitoes could get in a car, boat, etc. and travel long distances(100-102). They are believed to have originally come from Africa so they can travel far. Oxitec must reassess and include this new information.

Female *Aedes aegypti* may survive to adulthood due to a tendency to breed indoors where pet food with tetracyclines are present and where pet water medicated with tetracyclines are present. Manure from animals, such as pets fed food with tetracycline, chickens in the Keys, livestock in California, or fertilizer, could allow females to survive to adulthood, especially considering overwhelming peer reviewed evidence indicating that *Aedes aegypti* do not prefer to breed in clean water.

Oxitec calls this a, *"Sterile Insect Technique"* claiming GE mosquitoes can only reach adulthood in the presence of a specific dose of tetracycline.(9) However, a study suggests 15% of the larvae produced survived to adulthood when those larvae were in the presence of cat food likely contaminated with tetracycline(12) and Oxitec's own study found 18.4% survival in such conditions.(67) An employee of the Florida Keys Mosquito Control District admitted that Aedes aegypti larvae have been found in pet dishes(9), suggesting this scenario would likely occur if released in the Keys or California. The EPA replied, *"As the trial areas are expected to be in urbanized areas, the presence of pets and their food, such as cat food that may originate from organs/meat from antibiotic treated husbandry animals, is likely. However, cat food is not believed to be a plausible source of tetracycline exposure for OX5034 mosquitoes in the environment as it would require that adequate levels of tetracycline would be found in the cat food. This would require a number of steps: that the cat food be found in a container to create a high enough concentration of tetracycline to rescue OX5034 females, that the container also hold adequate levels of water for mosquito development, and that these conditions be maintained over a period of at least 8-10 days for larval and pupal development. In addition, exposure to sunlight would result in aqueous photolysis, so to maintain adequate tetracycline levels the cat food container would have to remain in the shade. For the reasons cited for cat food, other meat-based pet foods are not considered to be plausible sources of tetracycline exposure."* Yet, the conditions would in fact be common

in the Florida Keys and parts of California. Water is often added to pet food and the Florida Keys, and parts of California, are often areas that are only used temporarily throughout the year, such as only in the fall and winter as vacation homes. As this is a common occurrence, it is often common for people to leave vacation homes without cleaning up pet dishes. This would allow for the right amount of time for *Aedes aegypti* to deposit eggs and those mosquitoes survive to adulthood. Peer reviewed evidence indicates that *Aedes aegypti* often deposit eggs indoors in homes.(126,127) In fact, peer reviewed evidence, *"found that the infestation of indoor containers by Ae. aegypti was greater than outdoor containers."* and concluded, *"Many studies have demonstrated that Ae. aegypti rest indoors,[38,39] feed indoors[40] and oviposit indoors.[14]."*(Reference 128) Therefore, sunlight would not result in aqueous photolysis as *Aedes aegypti* often breed indoors and in pet food bowls.

Since tetracyclines are also found in the environment, there are other ways OX5034 x wild *Aedes aegypti* larvae could be exposed such as tetracycline being found in sewage, drinking water, animal feces, liquid manure and even tetracycline applied as a veterinary medicine in the water bowls of livestock and pets placed indoors or outdoors. Again, tetracyclines are often added to pet's drinking water and as homes in the Florida Keys and California are often vacation homes that are unoccupied for months at a time, it is certainly possible, and likely, for water medicated with tetracyclines to be left out for months.

It is claimed, by Oxitec, that *Aedes aegypti* preferred to deposit their larvae in clean water(9), but overwhelming peer reviewed evidence suggests *Aedes aegypti* are equally or more attracted to water with certain materials in it than they are clean water.(13,16,17,18,19) These include containers with algae, decaying leaves, infusions of various leaves and grasses(16, 17, 18), even horse manure(19) and sewage water.(160). Studies also suggest *Aedes aegypti* larvae can develop in septic tanks, sewage treatment plants and cesspits.(14,15,113,114,115)

Since tetracycline can be found in manure(92) this poses another risk of tetracycline exposure by Oxitec's mosquitoes. Although the EPA replied, *"Thus, cattle, chicken or other husbandry animal manures, which may contain pass-through antibiotics, are not expected to be present in significant quantities."* This is inaccurate as the Florida Keys has a large chicken population located in the urban areas.(155) Pets also exist in urban areas and since pets are often fed foods containing tetracyclines their feces will also contain tetracyclines. Manures such as chicken or cow manures will also be used as fertilizers in these areas.

Oxitec uses a diet supplemented with 30 µg/ml of the tetracycline to rear its mosquitoes in the lab. The tetracycline derivatives oxytetracycline (OTC) and doxycycline (DOX, used to prevent malaria) could also allow the GE mosquitoes to breed. Oxytetracycline can be found at concentrations above 500 µg/g in animal manure and doxycycline at up to 78516.1 µg/kg dry weight in broiler manure, which is likely to be more than enough to inactivate the killing mechanism.(116, 117)

Oxitec then states for the amount of tetracyclines needed to have high survival rates, they were unable to find these levels in sewage in the scientific literature throughout the world.(9) However, studies do exist suggesting this level exists in slurry samples(20), tetracycline at these levels are in some municipal sewage(21), and prescribed doses of tetracycline for medicating animal drinking water(22).

While Oxitec attempted to argue that tetracyclines degrade rapidly when exposed to heat or sunlight(9), a study suggests that it can take 5 months for tetracyclines to degrade just 50% in liquid manure(20). Oxitec also neglected to realize that most people will likely put drinking water medicated with tetracycline intended for their animals, in shaded areas like a porch, and it is believed shaded areas are

where *Aedes aegypti* most often place their eggs.(23) Peer reviewed evidence also indicates that *Aedes aegypti* often deposit eggs indoors in homes as well where the temperature is controlled and sunlight isn't a factor.(126,127)

Tetracycline levels required for female offspring of OX5034 males mated with wild females, in the Keys or California, to survive to adulthood may be lower than OX5034 bred with lab strains.

Oxitec appears to be speculating as to the amount of tetracycline required based on past breeding experiments and not experiments using the wild *Aedes aegypti* found in the Keys or California. It is possible that when Oxitec males breed wild *Aedes aegypti* females found in the Keys and/or California it can alter the amount of tetracycline required for the female offspring to survive to adulthood. Therefore, Oxitec must conduct studies to determine the amount of tetracycline that would be required for the offspring to survive to adulthood when Oxitec males breed wild *Aedes aegypti* females found in the Keys and California. Since reproducibility is one of the main principles of the scientific method, these studies must be reproduced by independent experts. This can be done through programs such as PLOS ONE's Reproducibility Initiative.(76) Once Oxitec has conducted these studies and they are replicated by independent experts, then Oxitec must reassess and include this new information. Until Oxitec has conducted such studies and they are replicated by independent experts their GE mosquitoes cannot be considered safe.

Current levels of tetracyclines in the environment in the Florida Keys and California have not been tested. Lowest level of tetracyclines required for female survival has not been published and cannot be replicated.

Since tetracycline in the environment could cause female Aedes aegypti to survive to adulthood, there is a risk that female OX5034 may survive to adulthood after eggs are placed in an area with tetracycline present. Oxitec must provide data, preferably from peer reviewed studies, that recent samples of soil, pet food, septic tanks, cesspits, manure, etc. in the experimental areas have been tested for tetracycline, chlortetracycline, doxycycline, oxytetracycline, etc. They must also provide the lowest dosage recorded to allow a female to survive to adulthood so that independent scientists can attempt to replicate their results. This is important as It is possible for the females that mate with the OX5034 or the OX5034 males to leave the test area and travel to an area such as a citrus grove in Florida where oxytetracycline is sprayed on citrus crops. The current test area has a 500 meter buffer zone. Data from peer reviewed studies indicate that Aedes aegypti can fly significantly further than 500 meters, are routinely transported by vehicles and Aedes aegypti from South Florida have traveled as far away as the Netherlands.(96,100,103)

Claims that no females survive to adulthood are based on breeding with laboratory strains and Brazilian *Aedes aegypti* and not wild *Aedes aegypti* in Florida and California.

The claim of no female offspring surviving to adulthood is based on past breeding experiments and not experiments using the wild *Aedes aegypti* found in the Keys or California. It is possible that when Oxitec males breed with wild *Aedes aegypti* females found in the Keys or California, it can alter the amount of female offspring that survive as target pest populations may have genetic background components which provide resistance to lethal systems.(170) Therefore, Oxitec must conduct studies to determine the amount of female offspring that survive to adulthood when Oxitec males breed wild *Aedes aegypti* females found in the Keys and California. Since reproducibility is one of the main principles of the scientific method, these studies must be reproduced by independent experts. This can be done through programs such as PLOS ONE's Reproducibility Initiative.(76) Once Oxitec has conducted these necessary studies and they are replicated by independent experts, then Oxitec must reassess and include this new information. Until Oxitec has conducted such studies and they are replicated by independent experts their GE mosquitoes cannot be considered safe.

Oxitec's previous studies have been too small to determine that no females will be released. Data from Brazil is not sufficient.

Although the amount of female offspring surviving to adulthood could increase if any larvae are exposed to tetracycline via cat food, medicated water, etc., the percentage of surviving GM insects, including biting females, could also increase if resistance to the genetic killing mechanism evolves over time: for example, genetic mutations in the insects which allow the GM insects to survive and breed successfully could be rapidly selected for during mass production.(69, 70) Peer reviewed evidence suggests that mutations are likely to cause some female offspring to survive to adulthood despite Oxitec's claims.(125) Testing should be done in the laboratory using numbers of mosquitoes equivalent or greater to the amount planned for use in the Florida Keys and California to determine that no females will survive to adulthood in these test areas. Small samples of hundreds or thousands of mosquitoes cannot be extrapolated to draw conclusions that no females will be released when millions of OX5034 are released. Since traps do not capture all *Aedes aegypti*, data from traps used in previous releases in Brazil are not sufficient to determine no females survived to adulthood in the Brazilian experiments or will not survive to adulthood in the Florida Keys or California experiments, especially considering the large number of OX5034 that will be released. Since reproducibility is one of the main principles of the scientific method, these studies must be reproduced by independent experts. This can be done through programs such as PLOS ONE's Reproducibility Initiative.(76) Once Oxitec has conducted these necessary studies and they are replicated by independent experts, then Oxitec must reassess and include this new information. Until Oxitec has conducted such studies and they are replicated by independent experts their GE mosquitoes cannot be considered safe.

Biological containment of OX5034 is impossible.

Since tetracycline is not likely to be eliminated from pet food or the environment in the near future, there is a significant chance that these Oxitec mosquitoes will survive and therefore biological containment is an impossibility at this point. Oxitec must reassess and include this new information.

Even in the absence of tetracyclines Oxitec's mosquitoes are likely to remain in the environment due to the offspring that survive to adulthood without exposure to tetracycline. The Food and Agriculture Organization of the United Nations agrees on their website stating, *"The transgenic approaches instead can have potentially unforeseen consequences because the released insects are not sterile and therefore will reproduce and become established."*(91)

Feeding trials of insectivores that consume *Aedes aegypti* over 48 months have not been conducted. Feeding trials using predators who consume prey that have consumed OX5034 or its novel transgenic proteins have not been done. Evidence for the safety of insectivores that will consume OX5034 are virtually non-existent. No multigenerational or transgenerational studies have been done for any species consuming OX5034 or its novel transgenic proteins. Endangered species have not been considered. No monitoring of animal health during the releases is being done and therefore adverse effects to animals as a result of this experiment would go unnoticed.

Nine species of dragonflies and three species of damselflies found in the Keys can eat mosquitoes, carnivorous plants like small butterwort(194), lizards like the green anole(195) and amphibians like green tree frog tadpoles(186), etc. all can eat mosquitoes. The EPA claims, *"Due to the preferred larval habitat of Ae. aegypti mosquitoes, exposure to vertebrate predators is expected to be limited, therefore also limiting the role Ae. aegypti play in the predator diet."* However, this ignores that dragonfly larvae, for example, may consume large amounts of *Aedes aegypti* larvae and(158,159) tadpoles can also consume large amounts of *Aedes aegypti* eggs(185) and larvae.(192) For example, the green tree frog native to Florida consumes mosquito larvae.(186) Salamander larvae also consume large amounts of mosquito larvae at a level comparable to the mosquitofish.(188,191)

Dragonfly larvae, salamander larvae and tadpoles are often found in the same larval habitat as *Aedes aegypti*, in fact peer reviewed evidence indicates *Aedes aegypti* prefer to lay their eggs in water sources with tadpoles of the genera *Polypedates, Bufo, Ramanella, Euphlyctis and Hoplobatrachus* in them.(185) Since salamander larvae and tadpoles are confined to those larval habitats, unable to walk or hop out, they will consume large amounts of *Aedes aegypti* as a primary food source. This is especially important since this is a large consumption of OX5034 early in their development. As the EPA mentions, *"as many of the aquatic insects that may consume OX5034 larvae are larvae themselves and thus more susceptible to even low-level toxins, additional certainty regarding the lack of toxicity to aquatic insect larvae could be gained through a larval feeding study prior to a Section 3 registration. "* However, larval feeding studies should be conducted before any further experimentation especially considering many of these larvae that will consume *Aedes aegypti* are endangered species. For example tiger salamander tadpoles are known for consuming large amounts of mosquito larvae(189) and the California tiger salamander is an endangered species.(190) While the EPA claims, *"it does not appear that the salamanders noted above feed significantly on Ae. aegypti larvae"*, the EPA provides no evidence for such a claim. In fact, the EPA notes, *"the Tiger Salamander, Ambystoma tigrinum, was found to readily consume mosquito*

(Culicidae, species not identified) larvae based on 26% of analyzed stomach samples containing remnants of larvae (Brodman and Dorton 2006)." If the species was not identified in this study, the EPA has no basis to claim the tiger salamander does not feed significantly on Aedes aegypti, considering according to Brodman and Dorton, *"mosquitoes were third most abundant prey taxon and were found in 26% of the tiger salamander larvae ranking second behind cladocerans ".* This would certainly indicate that tiger salamanders feed significantly on mosquito larvae. In the study by Brodman and Dorton it states, *"mosquito larvae were a preferred prey"* and, *"We observed that tiger salamander larvae can eat a large number of mosquito larvae in short periods of time."*

Another risk is the bioaccumulation of the transgenic proteins. Other transgenic proteins have been known to bioaccumulate and harm predators that eat the organisms that consume the transgenic proteins.(197,198) This poses a risk for organisms higher up in the food web that eat the organisms that consume the OX5034 larvae, pupae or adults and not just the organisms that directly consume the OX5034. This becomes an even greater risk for organisms such as frogs or salamanders who would consume OX5034 early in their life cycle and then as adults eat organisms, such as dragonflies, that also consumed OX5034.

Even organisms that have not consumed OX5034 larvae, pupae, or adults may still be consuming these transgenic proteins. Transgenic proteins can adsorb to the surface of algae, cyanobacteria and macrophytes that are then consumed by higher organisms up the food web, which then bioaccumulate and are then consumed by even higher organisms up the food web.(199) In this way nearly every endangered species may be consuming these transgenic proteins unique to OX5034. Testing must be done on predators of *Aedes aegypti* that may consume OX5034 as larvae, pupae or adults to see if bioaccumulation of these novel transgenic proteins occurs. If bioaccumulation does occur, feeding trials of predators who consume these organisms that consume OX5034 must be done to assess toxicity. Also organisms that may consume the transgenic proteins by other means, via algae, cyanobacteria and macrophytes consumption, must be tested for bioaccumulation. Predators that consume those organisms that have consumed those transgenic proteins must also be tested for toxicity.

Although Oxitec claimed for OX513A, *"The Stock Island Tree Snail is the only species found in the physical vicinity of the proposed trial site."*(121) they ignored that mosquitoes are capable of getting in a car, boat, or other means of transport(100-102), these mosquitoes are capable of leaving the physical vicinity of the proposed trial site and could even travel as far as another country(103,104). Therefore, Oxitec must consider this and, at a minimum, assess risk for all threatened, endangered, or candidate species that were identified in Monroe County. As Oxitec points out, *"National Key Deer Refuge headquarters is located on Big Pine Key, which is 100-miles south of Miami and 30 miles north of Key West on Highway US-1, and 26 miles from Key Haven"*. Since Oxitec's mosquitoes can enter a vehicle and that vehicle can easily travel 26 miles, this type of scenario is very likely. Oxitec must then consider this is very likely and at a minimum assess risk for all threatened, endangered, or candidate species that were identified in Monroe County. For example, endangered or threatened reptiles in Florida Keys that consume mosquitoes, eggs, larvae, pupae or adults, or eat other species which consume mosquitoes include *Eumeces egregius egregius* and *Eumeces egregius insularis*. Endangered or threatened birds in Florida Keys that consume mosquitoes, eggs, larvae, pupae or adults, or consume other species which consume mosquitoes include *Ammodramus maritimus mirabilis*, *Vermivora bachmanii*, *Calidris canutus rufa* and *Charadrius melodus*. Oxitec must reassess and include this new information. It should be noted that due to the limited time of only 30 days to comment we were unable to do an exhaustive search for all

endangered, threatened or candidate species in the Florida Keys that may consume mosquitoes, eggs, larvae, pupae or adults, or eat other species which consume mosquitoes. Therefore, our list of endangered and threatened species should be considered severely lacking and many more species are likely applicable which are not mentioned here.

Oxitec must also assess the risk of all endangered species in the state of California. Besides *Ambystoma californiense* a number of endangered amphibians in California are also very likely to consume large amounts of *Aedes aegypti*, eggs, larvae, pupae or adults, or consume other species which consume mosquitoes, these include, but are not limited to: *Ascaphus truei, Batrachoseps sp, Batrachoseps campi, Batrachoseps pacificus pacificus, Batrachoseps relictus, Batrachoseps simatus, Batrachoseps stebbinsi, Bufo canorus, Bufo exsul, Bufo microscaphus californicus, Ensatina eschscholtzii croceator, Ensatina eschscholtzii klauberi, Hydromantes sp, Hydromantes brunus, Hydromantes platycephalus, Hydromantes shastae, Plethodon elongatus, Plethodon stormi, Rana aurora aurora, Rana aurora draytoni, Rana boylii, Rana cascadae, Rana muscosa, Rana pretiosa, Rana yavapaiensis, Rhyacotriton variegatus, Scaphiopus hammondii*. Considering, for example, that several *Bufo* are endangered in California and *Aedes aegypti* prefer to lay their eggs in water sources with tadpoles of the genera *Bufo* in them(185) this means that the subjects used for toxicity testing by Oxitec are inadequate to assess the safety of amphibians that will almost definitely consume large amounts of OX5034 if released. Toxicity testing with amphibians consuming OX5034 is absolutely necessary considering the risk to endangered species.

Endangered or threatened reptiles in California that may consume mosquitoes, eggs, larvae, pupae or adults, or eat other species which consume mosquitoes include, but are not limited to: *Anniella pulchra nigra, Anniella pulchra pulchra, Clemmys marmorata marmorata, marmorata, Cnemidophorus hyperythrus, Cnemidophorus tigris multiscutatus, Coleonyx switaki, Coleonyx variegatus abbotti, Elgaria panamintina, Eumeces skiltonianus interparietalis, Heloderma suspectum cinctum, Sceloporus graciosus graciosus, Sceloporus graciosus vandenburgianus, Uma notata notata, Xantusia henshawi gracilis, Xantusia vigilis sierrae*. Toxicity testing with reptiles consuming OX5034 is absolutely necessary considering the risk to endangered species.

Endangered or threatened insects in California that may consume mosquitoes, eggs, larvae, pupae or adults, or eat other species which consume mosquitoes include, but are not limited to: *Capnia lacustra, Ammopelmatus kelsoensis, Ammopelmatus muwu, Macrobaenetes kelsoensis, Ambrysus funebris, Pelocoris shoshone, Agabus rumppi, Chaetarthria leechi, Hydroporus hirsutus, Hydroporus leechi, Hydroporus simplex, Hygrotus curvipes, Hygrotus fontinalis.*

Endangered or threatened birds in California that may consume mosquitoes, eggs, larvae, pupae or adults, or eat other species which consume mosquitoes include, but are not limited to: *Agelaius tricolor, Aimophila ruficeps canescens, Amphispiza belli belli, Chlidonias niger, Geothlypis trichas sinuosa, Histrionicus histrionicus, Ixobrychus exilis hesperis, Laterallus jamaicensis, Plegadis chihi, Toxostoma lecontei macmillanorum.*

It should be noted that due to the limited time of only 30 days to comment, and the extensive area, the entire state of California as the specific counties were not identified, we were were unable to do an exhaustive search for all endangered, threatened or candidate species in California that may consume mosquitoes, eggs, larvae, pupae or adults, or eat other species which consume mosquitoes. Therefore, our list of endangered and threatened species should be considered severely lacking and many more species are likely applicable which are not mentioned here.

Oxitec states in SECTION G PROPOSED EXPERIMENTAL PROGRAM that, *"Season of Application: May-December (but could be deployed any time of year)"*. Oxitec has not provided adequate evidence to conclude there is no toxicity for insectivores in the Keys or California, especially for a duration of potentially all year for at least 24 months, and now even longer in Florida for potentially another 2 years. The only studies Oxitec has provided are a 14 day acute toxicity study using *Poecilia reticulata* and a 96 hour study using *Pacifastacus leniusculus*. These studies are of insufficient duration and include insufficient parameters to assess subchronic and chronic toxicity or carcinogenicity. These studies also have little to no relevance for insects, lizards, amphibians, carnivorous plants, etc. that may consume OX5034 as larvae, pupae, or adults. Oxitec must therefore perform toxicity studies using insectivores present in the Keys and California for a duration of at least 48 months, or the life of the subject if the subject does not live for 48 months. These toxicity studies should not be limited to mortality, appearance, size, and behavior, but should include examination of all major organ systems, including histological examination of organs as well as all other health parameters typical of toxicity studies. Multigenerational exposure, as well as transgenerational effects must also be considered since a large number of environmental factors have been shown to promote the epigenetic transgenerational inheritance of disease or phenotypic variation in a variety of different species, including humans.(1) Until Oxitec has conducted such studies their GE mosquitoes cannot be considered safe for any insectivores in the Keys.(112)

Oxitec claims, ""*The consequences of escape, survival, and establishment of OX513A in the environment have been extensively studied: data and information from those studies indicates that there are unlikely to be any adverse effects on non-target species, including humans. There are also unlikely to be any adverse effects on foreign countries or the global commons. Risk of establishment or spread has been determined to be negligible. The trial is short in duration and any unanticipated adverse effects are unlikely to be widespread or persistent in the environment. Most importantly, the status of the environment is restored when releases are stopped (i.e., the released mosquitoes all die, and the environment reverts to the pretrial status).*"(121) While this claim was made about OX513A it is also applied to OX5034. There is no data provided to support this claim, hence it is an unsubstantiated claim at best and cannot be assumed to be true without data.(112) Oxitec has withdrew an application for another of their genetically engineered insects when regulators asked questions which Oxitec could not answer.(52) Oxitec told Olive Oil Times that Spain's National Biosafety Commission requested that predator studies be held. Oxitec stated they would conduct the studies requested.(36) If Oxitec is willing to conduct these studies for Spain they must be willing to conduct them for the U.S. as well. Since reproducibility is one of the main principles of the scientific method, these studies must be reproduced by independent experts. This can be done through programs such as PLOS ONE's Reproducibility Initiative.(76) Once Oxitec has conducted these necessary studies and they are replicated by independent experts, then Oxitec must reassess and include this new information. Until Oxitec has conducted such studies and they are replicated by independent experts their GE mosquitoes cannot be considered safe.

No studies on mammals have been conducted. Evidence for human safety for those that consume OX5034 or its transgenic proteins, directly or indirectly, are virtually non-existent. No studies on the bioaccumulation of transgenic proteins have been done. Feeding trials using mammals who consume prey that have consumed OX5034 or its novel transgenic

proteins have not been done. No multigenerational or transgenerational studies have been done for any mammal species consuming OX5034 or its novel transgenic proteins. Endangered species have not been considered. No monitoring of human or mammal health during the releases is being done and therefore adverse effects to humans or animals as a result of this experiment would go unnoticed.

If GE mosquito larvae are deposited in pet dishes, a dog, cat, etc. may drink the water and consume some larvae in the process. A young child might even drink from a cup left inside or outside with OX5034 x wild *Aedes aegypti* larvae in it. So, what happens if the transgene is consumed by any or all of these species? Nobody knows, because there have been no studies published on the subject. Without testing of actual species in the Keys and California, Oxitec is basing their assessment on speculation and not science.(112)

Another risk is the bioaccumulation of the transgenic proteins. Other transgenic proteins have been known to bioaccumulate and harm predators that consume the organisms that consume the transgenic proteins.(197,198) This poses a risk for organisms higher up in the food web that eat the organisms that consume the OX5034 larvae, pupae or adults and not just the organisms that directly consume the OX5034. This becomes an even greater risk for organisms such as frogs or salamanders who would consume OX5034 early in their life cycle and then as adults consume organisms, such as dragonflies, that also consumed OX5034. This then poses an even greater risk to mammals, such as humans, that may consume an animal such as a frog.

However, even organisms that have not consumed OX5034 larvae, pupae, or adults may still be consuming these transgenic proteins. Transgenic proteins can adsorb to the surface of algae, cyanobacteria and macrophytes that are then consumed by higher organisms up the food web, which then bioaccumulate and are then consumed by even higher organisms up the food web.(199) In this way nearly every endangered mammal may be consuming these transgenic proteins unique to OX5034. Testing must be done on predators of *Aedes aegypti* that may consume OX5034 as larvae, pupae or adults to see if bioaccumulation of these novel transgenic proteins occurs. If bioaccumulation does occur, feeding trials of mammals who eat these organisms that consume OX5034 must be done to assess toxicity. Also organisms that may consume the transgenic proteins by other means, via algae, cyanobacteria and macrophytes consumption, must be tested for bioaccumulation. Mammals that eat those organisms that have consumed those transgenic proteins must also be tested for toxicity.

Mammals in the Florida Keys such as the bat *Molossus molossus*(196) eat mosquitoes as well as other insects, like dragonflies, that consume mosquitoes. Endangered or threatened mammals in California that consume mosquitoes, eggs, larvae, pupae or adults, include, but are not limited to: *Euderma maculatum, Eumops perotis californicus, Idionycteris phyllotis, Macrotus californicus, Myotis ciliolabrum, Myotis evotis, Myotis lucifugus occultus, Myotis thysanodes, Myotis velifer, Myotis volans, Myotis yumanensis, Plecotus townsendii townsendii.* Toxicity testing with mammals consuming OX5034 is absolutely necessary considering the risk to endangered species.

Oxitec states in SECTION G PROPOSED EXPERIMENTAL PROGRAM that, *"Season of Application: May-December (but could be deployed any time of year)"*. Oxitec has not provided adequate evidence to conclude there is no toxicity for insectivores in the Keys or California, especially for a duration of

potentially all year for at least 24 months, and now even longer in Florida for potentially another 2 years. The only studies Oxitec has provided are a 14 day acute toxicity study using *Poecilia reticulata* and a 96 hour study using *Pacifastacus leniusculus*. These studies are of insufficient duration and include insufficient parameters to assess subchronic and chronic toxicity or carcinogenicity. These studies have little to no relevance for mammals, including humans that may consume or otherwise be exposed to OX5034. Oxitec must therefore perform toxicity studies using insectivores present in the Keys and California for a duration of at least 48 months, or the life of the subject if the subject does not live for 48 months. These toxicity studies should not be limited to mortality, appearance, size, and behavior, but should include examination of all major organ systems, including histological examination of organs as well as all other health parameters typical of toxicity studies. Multigenerational exposure, as well as transgenerational effects must also be considered since a large number of environmental factors have been shown to promote the epigenetic transgenerational inheritance of disease or phenotypic variation in a variety of different species, including humans.(1) Until Oxitec has conducted such studies their GE mosquitoes cannot be considered safe for any insectivores in the Keys.(112)

Oxitec should also conduct feeding trials using rodents and non-rodents comparable to humans to assess toxicity as it may relate to humans, since humans may also accidentally swallow Oxitec's mosquitoes. The studies should also be for the life of the rodents and 48 months in duration for non-rodents and should not be limited to mortality, appearance, size, and behavior, as their previous studies are limited to, but should include examination of all major organ systems, include histological examination of organs as well as all other health parameters typical of chronic toxicity/carcinogenicity studies. Multigenerational exposure, as well as transgenerational effects must also be considered since a large number of environmental factors have been shown to promote the epigenetic transgenerational inheritance of disease or phenotypic variation in a variety of different species, including humans.(1) Until Oxitec has conducted such studies their GE mosquitoes cannot be considered safe for any mammals in the Keys or California.(112) Since reproducibility is one of the main principles of the scientific method, these studies must be reproduced by independent experts. This can be done through programs such as PLOS ONE's Reproducibility Initiative.(76) Once Oxitec has conducted these necessary studies and they are replicated by independent experts, then Oxitec must reassess and include this new information. Until Oxitec has conducted such studies and they are replicated by independent experts their GE mosquitoes cannot be considered safe.

Oxitec claims, *"The consequences of escape, survival, and establishment of OX513A in the environment have been extensively studied: data and information from those studies indicates that there are unlikely to be any adverse effects on non-target species, including humans. There are also unlikely to be any adverse effects on foreign countries or the global commons. Risk of establishment or spread has been determined to be negligible. The trial is short in duration and any unanticipated adverse effects are unlikely to be widespread or persistent in the environment. Most importantly, the status of the environment is restored when releases are stopped (i.e., the released mosquitoes all die, and the environment reverts to the pretrial status)."*(121) While this claim was made about OX513A it is also applied to OX5034. There is no data provided to support this claim, hence it is an unsubstantiated claim at best and cannot be assumed to be true without data.(112) Oxitec has withdrew an application for another of their genetically engineered insects when regulators asked questions which Oxitec could not answer.(52) Oxitec told Olive Oil Times that Spain's National Biosafety Commission requested that

predator studies be held. Oxitec stated they would conduct the studies requested.(36) If Oxitec is willing to conduct these studies for Spain they must be willing to conduct them for the U.S. as well.

The proteins expressed by the transgenes may be toxic.

Although Oxitec claims, *"tTA and its variants, such as tTAV, have been used in fungi, rodents, plants, and mammalian cultures with no known non-target adverse effects on the environment or human health"*(121) signs of toxicity(71) and neurotoxicity(72) have been reported in mice expressing the tTA protein. Other mice studies have detected adverse effects on the lung.(118) Oxitec later claims, *"Although some potential symptoms of toxicity have been reported in transgenic mice expressing high levels of tTA or its variants (Whitsett and Perl, 2006) other papers have observed no apparent toxicity"* However, this is not evidence to counter the potential symptoms of toxicity that have been observed. Oxitec must therefore reassess and include this new information as valid and not simply dismiss it. Oxitec should therefore attempt to replicate these studies finding toxicity and until Oxitec has conducted such studies and they are replicated by independent experts their GE mosquitoes cannot be considered safe.

DsRed2 has cytotoxic effects on bacteria(201) as well as human cells and this may increase in the presence of doxycycline.(202)

Gene transfer of transgenes from OX5034 to intestinal microbiota of insectivores consuming OX5034, humans accidentally consuming OX5034, or soil or aquatic bacteria may occur and has not been properly evaluated.

Although Oxitec claims, *"in the case of birds eating mosquitoes (and humans unintentionally swallowing them), animals do not incorporate DNA from their food into their genome."*(121), Oxitec appears unaware that gene transfer to intestinal microbiota from food has been observed from the consumption of GE food even in humans.(74) Since Oxitec was obviously unaware of this, they must conduct studies to determine if gene transfer to intestinal microbiota of insectivores in the Keys occurs. Even if it does not occur in the lab it may still occur in the environment. Therefore, Oxitec must conduct studies which introduce these genes into microbiota commonly found in insectivores in the Keys as well as microbiota found in humans to determine what impact gene transfer of these genes would have on these microbiota if it occurred. Since reproducibility is one of the main principles of the scientific method, these studies must be reproduced by independent experts. This can be done through programs such as PLOS ONE's Reproducibility Initiative.(76) Once Oxitec has conducted these necessary studies and they are replicated by independent experts, then Oxitec must reassess and include this new information. Until Oxitec has conducted such studies and they are replicated by independent experts their GE mosquitoes cannot be considered safe.

Oxitec also claims, *"several studies have addressed the fate of ingested DNA in mammals and birds, including attempts to detect recombinant DNA in chicken (Khumnirdpetch et al., 2001) or cows (Klotz and*

Einspanier, 1998) fed with glyphosate tolerant soybean and in pork (Weber and Richert, 2001) pigs (Klotz et al., 2002), dairy cows, beef steers, and broiler chicken (Einspanier et al., 2001; Flachowsky et al., 2000), all fed with recombinant Bacillus thuringiensis corn. In none of those studies was recombinant DNA detectable by PCR in various samples."(121) However, studies suggest that transgenes, from the consumption of GE foods, may transfer to the intestinal microbiota of humans.(74) Animal studies where animals have consumed GE foods suggest that some transgenes in GE foods may be able to transform oral bacteria(68). That some transgenes in GE foods may survive passage through the small intestine(63) and have been detected in feces.(62) The possibility that consumption of GE mosquitoes by insectivores may result in the transgene(s) being found in feces leads to potential risks such as soil microbes being exposed to the feces, and thus the transgene(s), and the potential for gene transfer from the transgene from feces to soil microorganisms. Even fragmented, or partially degraded, transgenes may be able to transform oral bacteria or soil bacteria(181) This is important as fragmented transgenes from OX5034 are likely to remain in the environment for a long enough period for transformation to occur. Animal studies, not included in Oxitec's assessment, have observed transgenes in blood, kidneys, liver, heart, muscle, brain and milk of animals fed GE foods as well as their offspring.(47-51,61,62) Since Oxitec appears unaware that the empirical evidence disagrees with their claim they must conduct studies to determine if transgenes, or fragments of transgenes, are found in insectivores found in the Keys that may consume them since the evidence clearly shows this occurs in other animals, etc. that consume genetically engineered foods. Since reproducibility is one of the main principles of the scientific method, these studies must be reproduced by independent experts. This can be done through programs such as PLOS ONE's Reproducibility Initiative.(76) Once Oxitec has conducted these necessary studies and they are replicated by independent experts, then Oxitec must reassess and include this new information. Until Oxitec has conducted such studies and they are replicated by independent experts their GE mosquitoes cannot be considered safe.

Laboratory reared *Aedes aegypti* may bioaccumulate heavy metals which are harmful to dragonflies and tadpoles which heavily consume *Aedes aegypti* in the wild.

Evidence indicates that *Aedes aegypti* can bioaccumulate heavy metals over several generations and consumption of the *Aedes aegypti* with high heavy metal content adversely impacts dragonflies that consume them.(165) Heavy metal exposure is also detrimental to tadpoles.(187) The EPA claims, *"Due to the preferred larval habitat of Ae. aegypti mosquitoes, exposure to vertebrate predators is expected to be limited, therefore also limiting the role Ae. aegypti play in the predator diet."* However, this ignores that dragonfly larvae, for example, may consume large amounts of *Aedes aegypti* larvae and(158,159) tadpoles can also consume large amounts of *Aedes aegypti* eggs(185) and larvae.(192) For example, the green tree frog native to Florida consumes mosquito larvae.(186) Salamander larvae also consume large amounts of mosquito larvae at a level comparable to the mosquitofish.(188,191) Dragonfly larvae, salamander larvae and tadpoles are often found in the same larval habitat as *Aedes aegypti*, in fact peer reviewed evidence indicates *Aedes aegypti* prefer to lay their eggs in water sources with tadpoles of the genera *Polypedates, Bufo, Ramanella, Euphlyctis and Hoplobatrachus* in them.(185) Since salamander larvae and tadpoles are confined to those larval habitats, unable to walk or hop out, they will consume large amounts of *Aedes aegypti* as a primary food source. This is especially important since this is a large

consumption of OX5034 early in their development. For example tiger salamander tadpoles are known for consuming large amounts of mosquito larvae(189) and the California tiger salamander is an endangered species.(190) While the EPA claims, *"it does not appear that the salamanders noted above feed significantly on Ae. aegypti larvae"*, the EPA provides no evidence for such a claim. In fact, the EPA notes, *"the Tiger Salamander, Ambystoma tigrinum, was found to readily consume mosquito (Culicidae, species not identified) larvae based on 26% of analyzed stomach samples containing remnants of larvae (Brodman and Dorton 2006)."* If the species was not identified in this study, the EPA has no basis to claim the tiger salamander does not feed significantly on Aedes aegypti, considering according to Brodman and Dorton, *"mosquitoes were third most abundant prey taxon and were found in 26% of the tiger salamander larvae ranking second behind cladocerans "*. This would certainly indicate that tiger salamanders feed significantly on mosquito larvae. In the study by Brodman and Dorton it states, *"mosquito larvae were a preferred prey"* and, *"We observed that tiger salamander larvae can eat a large number of mosquito larvae in short periods of time."*

Heavy metals may even bioaccumulate and harm predators that consume the organisms that consume the OX5034. This poses a risk for organisms higher up in the food web that eat the organisms that consume the OX5034 larvae, pupae or adults and not just the organisms that directly consume the OX5034. This becomes an even greater risk for organisms such as frogs or salamanders who would consume OX5034 early in their life cycle and then as adults consume organisms, such as dragonflies, that also consumed OX5034. However, even organisms that have not consumed OX5034 larvae, pupae, or adults may still be consuming these heavy metals. Heavy metals can adsorb to the surface of algae, cyanobacteria and macrophytes that are then consumed by higher organisms up the food web, which then bioaccumulate and are then consumed by even higher organisms up the food web.(200) In this way nearly every endangered species may be consuming these heavy metals from OX5034.

Besides *Ambystoma californiense* a number of endangered amphibians in California are also very likely to consume large amounts of *Aedes aegypti* including: *Ascaphus truei, Batrachoseps sp, Batrachoseps campi, Batrachoseps pacificus pacificus, Batrachoseps relictus, Batrachoseps simatus, Batrachoseps stebbinsi, Bufo canorus, Bufo exsul, Bufo microscaphus californicus, Ensatina eschscholtzii croceator, Ensatina eschscholtzii klauberi, Hydromantes sp, Hydromantes brunus, Hydromantes platycephalus, Hydromantes shastae, Plethodon elongatus, Plethodon stormi, Rana aurora aurora, Rana aurora draytoni, Rana boylii, Rana cascadae, Rana muscosa, Rana pretiosa, Rana yavapaiensis, Rhyacotriton variegatus, Scaphiopus hammondii*. Considering, for example, that several *Bufo* are endangered in California and *Aedes aegypti* prefer to lay their eggs in water sources with tadpoles of the genera *Bufo* in them(185) this means that some amphibians will almost definitely consume large amounts of OX5034 if released.

Endangered or threatened reptiles in California that may consume mosquitoes, eggs, larvae, pupae or adults, or eat other species which consume mosquitoes include, but are not limited to: *Anniella pulchra nigra, Anniella pulchra pulchra, Clemmys marmorata marmorata, marmorata, Cnemidophorus hyperythrus, Cnemidophorus tigris multiscutatus, Coleonyx switaki, Coleonyx variegatus abbotti, Elgaria panamintina, Eumeces skiltonianus interparietalis, Heloderma suspectum cinctum, Sceloporus graciosus graciosus, Sceloporus graciosus vandenburgianus, Uma notata notata, Xantusia henshawi gracilis, Xantusia vigilis sierrae*. Toxicity testing with reptiles consuming OX5034 is absolutely necessary considering the risk to endangered species.

Endangered or threatened insects in California that may consume mosquitoes, eggs, larvae, pupae or adults, or eat other species which consume mosquitoes include, but are not limited to: *Capnia lacustra, Ammopelmatus kelsoensis, Ammopelmatus muwu, Macrobaenetes kelsoensis, Ambrysus funebris, Pelocoris shoshone, Agabus rumppi, Chaetarthria leechi, Hydroporus hirsutus, Hydroporus leechi, Hydroporus simplex, Hygrotus curvipes, Hygrotus fontinalis.*

Endangered or threatened birds in California that may consume mosquitoes, eggs, larvae, pupae or adults, or eat other species which consume mosquitoes include, but are not limited to: *Agelaius tricolor, Aimophila ruficeps canescens, Amphispiza belli belli, Chlidonias niger, Geothlypis trichas sinuosa, Histrionicus histrionicus, Ixobrychus exilis hesperis, Laterallus jamaicensis, Plegadis chihi, Toxostoma lecontei macmillanorum.*

It should be noted that due to the limited time of only 30 days to comment, and the extensive area, the entire state of California as the specific counties were not identified, we were were unable to do an exhaustive search for all endangered, threatened or candidate species in California that may consume mosquitoes, eggs, larvae, pupae or adults, or eat other species which consume mosquitoes. Therefore, our list of endangered and threatened species should be considered severely lacking and many more species are likely applicable which are not mentioned here.

While heavy metal bioaccumulation over generations may not be a great concern for wild *Aedes aegypti*, where *Aedes aegypti* breed in different containers and consume different feeds over several generations, this is a concern for laboratory reared *Aedes aegypti* that will be exposed to the same environment and likely consume the same feed, be reared in the same habitats, etc. over generations. This is especially important because OX5034 mothers are fed on horse blood, and horse blood can contain heavy metals.(172,173) Therefore, the OX5034 must be tested for heavy metals.

The release of millions of OX5034 males may adversely impact native pollinators.

Native pollinators in the Florida Keys and California often rely on nectar as a food source. The addition of millions of OX5034 which also consume nectar is adding environmental resistance in the form of increased competition for food, and this could affect the biotic potential of pollinator populations.

The OX5034 release does not have redundant safety measures like similar experiments that release male *Aedes aegypti*

Current control methods do not include the release of millions of *Aedes aegypti* which could potentially increase the risk of mosquito-borne diseases. If the OX5034 mates with wild females harboring diseases, the male can then acquire diseases such as Zika from the infected female through venereal transmission.(139,140,141) Even when male *Aedes aegypti* are released in experiments there are built in redundant safety measures with those techniques as we would expect when dealing with diseases. OX5034 doesn't have those safety measures. If experiments used Wolbachia or IIT/SIT then this wouldn't

happen. There wouldn't be 50% of offspring surviving to spread diseases and/or the mosquitoes would be largely resistant to diseases like dengue, Zika, chikungunya, yellow fever, filarial nematodes, plasmodium, west nile virus, etc. anyway.(144,145) This makes Oxitec's proposal riskier, with regards to disease spread, than current control methods and similar experiments. Oxitec attempts to dismiss this fact by stating, *"released mosquitoes are disease-free as they are maintained in conditions and with procedures that prevent contamination with virus and because the dengue virus takes a long time to develop in a mosquito to the point where it can be transmitted, shorter-lived females such as the OX513A females are less likely to pass on diseases."* However, their previous statement that the life expectancy, *"did not differ significantly from the non-GE laboratory strain coreleased as part of a comparative evaluation"* contradicts the claim that Oxitec's mosquitoes are less likely to pass on diseases. However, this life expectancy data is not based on the Florida Keys or California environments as no caged trials have been done to determine how long OX5034 survive in these environments, it is also only based on small numbers of mosquitoes and not the millions of mosquitoes Oxitec plans to release in the Keys and California. Therefore, without actual data from the Florida Keys or California it should not be assumed that these mosquitoes will have the same life expectancy if released in the Florida Keys or California. However, for homozygous OX5034 males reared off-doxycycline there was a median survival of 24 days according to data Oxitec presented to the EPA. Hemizygous OX5034 males reared off-doxycycline had a median survival of 44 days. However, for Zika *"four-five days was enough to spread the virus in the body of the mosquito, and four-five days of mating were enough for venereal transmission to occur."*(140) Which gives the OX5034 time to spread mosquito-borne diseases. Therefore, Oxitec must reassess and include the maximum potential life expectancy of males released, since this would give OX5034 males released ample time to spread mosquito-borne diseases.

In studies in Senegal for example, they found conflicting evidence for the ability of *Aedes aegypti* to transmit ZIKV.(46) Some *Aedes aegypti* were able to effectively transmit ZIKV and others were not. In another study, *Aedes aegypti* from Cape Verde and Kedougou showed the potential to transmit chikungunya in saliva but not those from Dakar.(42) Since Oxitec plans to release millions of male GE mosquitoes, if Oxitec's mosquitoes can more effectively transmit ZIKV, dengue, chikungunya or other mosquito borne diseases, via venereal transmission, compared to the wild *Aedes aegypti* in the Florida Keys or California, this would represent a greater risk to humans in the Keys or California than wild *Aedes aegypti.* Therefore, Oxitec must conduct studies to assess the ability of GE mosquitoes to effectively transmit ZIKV, dengue, chikungunya, yellow fever, and all other mosquito borne diseases spread by *Aedes aegypti*, in comparison to wild *Aedes aegypti* currently found in the test area. This must include venereal and transovarial transmission testing. The receipt of seminal fluid proteins that are transferred from males to females along with sperm during copulation may cause changes in host-seeking and feeding behavior(94). Therefore, Oxitec must conduct studies to determine if there are differences in seminal fluid proteins and sperm of their GE mosquitoes compared to wild male mosquitoes in the Florida Keys and if any differences are observed what impact this has on the host-seeking and feeding behavior of wild *Aedes aegypti* females that mate with Oxitec's GE male mosquitoes. Oxitec must also conduct studies for all mosquito-borne diseases *Aedes aegypti* is a vector for with regards to transovarial transmission of OX5034 x wild *Aedes aegypti* in the Florida Keys and in California compared to wild male x wild female *Aedes aegypti* in these locations. Since reproducibility is one of the main principles of the scientific method, these studies must be reproduced by independent experts. This can be done through programs such as PLOS ONE's Reproducibility Initiative.(76) Once Oxitec has conducted these necessary studies and they are replicated by independent experts, then Oxitec must reassess and include this new

information. Until Oxitec has conducted such studies and they are replicated by independent experts their GE mosquitoes cannot be considered safe.

There is some evidence that antibiotics may increase the transmission of dengue fever by *Aedes aegypti* mosquitoes.(120) This suggests that Oxitec's mosquitoes, reared on the antibiotic tetracycline, are better able to transmit dengue fever compared to wild *Aedes aegypti*. Therefore, Oxitec must conduct studies to assess the ability of GE mosquitoes to effectively transmit ZIKV, dengue, chikungunya, yellow fever, and all other mosquito borne diseases spread by *Aedes aegypti*, in comparison to wild *Aedes aegypti* currently found in the test area. Since reproducibility is one of the main principles of the scientific method, these studies must be reproduced by independent experts. This can be done through programs such as PLOS ONE's Reproducibility Initiative.(76) Once Oxitec has conducted these necessary studies and they are replicated by independent experts, then Oxitec must reassess and include this new information. Until Oxitec has conducted such studies and they are replicated by independent experts their GE mosquitoes cannot be considered safe.

If the lethality trait fails millions of OX5034 female *Aedes aegypti* will be released

Oxitec has failed to realize that if the lethality trait fails that would mean a greater potential number of *Aedes aegypti*, both male and female, since Oxitec would have released millions of *Aedes aegypti* and these mosquitoes will successfully mate and have offspring that survive to adulthood. According to the mutation rates some OX5034 females may be released.(125) Another possibility is that target pest populations may have genetic background components which provide resistance to lethal systems.(170) Therefore, male OX5034 x wild Florida Keys and California *Aedes aegypti* offspring must be tested for female offspring that may survive to adulthood, and not just a reliance on male OX5034 x laboratory strain *Aedes aegypti*, or wild *Aedes aegypti* from areas outside of the Florida Keys and California. An increase in mosquitoes and mating would likely lead to an increased risk of mosquito-borne diseases. The current control methods do not include purposely increasing the mating of *Aedes aegypti*, leading to an increase in population if the lethality trait fails, which makes Oxitec's proposal riskier, with regards to disease spread, than current control methods.

Risk from piggyBac transposable element

Oxitec claims, *"The piggyBac transposable element is a non-autonomous transposon isolated from the cabbage looper moth Trichoplusia ni, which has been well studied and used to transform a wide range of insect taxa: Diptera, Lepidopteran, Coleoptera (Handler, 2002; Jasinskiene et al., 1998; Koukidou et al., 2006; Kuwayama et al., 2006; Labbé et al., 2010; Tamura et al., 2000). A non-autonomous transposon, which has integrated into the genome, is prevented from moving within or outside the genome of its host because it does not encode or produce the associated transposase enzyme that is necessary for such movement. "*(121) However, Joe Cummins and Mae-Wan Ho state, *"transposase function can be supplied by a 'helper' transposon. Such helper transposons are ubiquitous."*(5)

They also state, *"the disabled vector carrying the transgene, even when stripped down to the bare minimum of the border repeats, was nevertheless able to replicate and spread, basically because the transposase function enabling the piggyBac inserts to move can be supplied by 'helper' transposons. Such helper transposons are potentially present in all genomes...Although each transposon has its own specific transposase enzyme that recognizes its terminal repeats, the enzyme can also interact with the terminal repeats of other transposons, and evidence suggest "extensive cross-talk among related but distinct transposon families" within a single eukaryotic genome"*(4) Oxitec must reassess and include this new information.

What is Oxitec missing?

1. Risk and benefit

According to the Declaration of Helsinki found on the FDA website:

"Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research."(38)

The objective of this trial is a reduction in mosquitoes, though this reduction has no impact on mosquito-borne diseases. Oxitec admitted during a town hall meeting in the Keys that, *"In terms of dengue transmission we have done 4 or 5 trials now, but those trials have been too small to be able to show any sort of effect on dengue."*(37) Since this trial is as small as other Oxitec trials there is no potential reduction in any mosquito-borne diseases either. So there is no likelihood that the populations in which the research is carried out stand to benefit from the results of the research.

Current control methods do not include the release of millions of *Aedes aegypti* which could potentially increase the risk of mosquito-borne diseases. This makes Oxitec's proposal riskier, with regards to disease spread, than current control methods. Therefore, the inherent risks and burdens to the subject outweigh the importance of the objective.

Current control methods also do not include the potential consumption of synthetic DNA sequences found in Oxitec's mosquito. Gene transfer to intestinal microbiota from food has been observed from the consumption of GE food in humans(74) and such a risk also exists for Oxitec's mosquitoes if swallowed. So, in this case, based on medical ethics found on the FDA website, the trial should not proceed.

2. Informed Consent

According to the Declaration of Helsinki found on the FDA website :

"In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing."(38)

According to the Nuremberg Code found on the U.S. Department of Health & Human Services website :

"The voluntary consent of the human subject is absolutely essential.

This means that the person involved should have legal capacity to give consent; should be so situated as to be able to exercise free power of choice, without the intervention of any element of force, fraud, deceit, duress, over-reaching, or other ulterior form of constraint or coercion; and should have sufficient knowledge and comprehension of the elements of the subject matter involved, as to enable him to make an understanding and enlightened decision. This latter element requires that, before the acceptance of an affirmative decision by the experimental subject, there should be made known to him the nature, duration, and purpose of the experiment; the method and means by which it is to be conducted; all inconveniences and hazards reasonably to be expected; and the effects upon his health or person, which may possibly come from his participation in the experiment.

The duty and responsibility for ascertaining the quality of the consent rests upon each individual who initiates, directs or engages in the experiment. It is a personal duty and responsibility which may not be delegated to another with impunity."(3)

A survey of Key Haven residents found 75% oppose GM mosquitoes.(66)

"There are a variety of ethical issues that are raised from the use of genetically modified insects," wrote Darryl Macer, who has published papers on the subject for the World Health Organization and in academic journals. *"But the most challenging may be the process of informed consent for individuals and communities. Each community or society needs to be given a chance to set consensus."*(35)

Mosquitoes are believed to frequently travel long distances via boat, automobile, etc.(100) and this is also likely true for Florida.(101). It is believed that the recent presence of *Aedes aegypti* in California was caused by commerce via air, railroad, or trucks traveling from the southern U.S.(102). Even *Aedes*

aegypti found as far away as the Netherlands are believed to have traveled there in airplane tires arriving from southern Florida.(103,104) With over 3 million visitors to the Florida Keys(25) a year, and numerous vehicles traveling in and out of the area, these genetically engineered mosquitoes escaping the test area is an extremely likely scenario. Since these mosquitoes will likely travel by vehicle to locations outside of the Florida Keys or California, and potentially other countries, the lack of informed consent by communities outside of the Keys and California creates ethical issues.(112) A national survey of 1,211 people found that when people were told about the technology and the risks, more people disagreed with the release of genetically engineered mosquitoes than agreed.(54) An online petition against the release of genetically engineered mosquitoes has over 237,000 signatures including many signatures from people outside of the Keys.(55) Since the Florida Keys has over 3 million visitors a year(25) the consent of not only the Keys residents, but also the consent of potential tourists must be considered.

Since the release of millions of additional males will likely increase the number of mated females and mated females live longer, take larger blood meals and may be more attracted to human odors which could increase the spread of mosquito-borne diseases(151,175) and these mated females require human blood to reproduce and have the offspring of the OX5034, a purpose of this experiment, this must be considered a human experiment. Since humans are directly involved and their blood is required for this experiment, humans are a subject in this experiment and this human experiment must abide by all requirements for an experiment on humans including, but not limited to, informed consent. So, in this case, based on medical ethics found on the FDA website, the trial should not proceed since the residents do not consent.

3. Misinformation and disease claims not approved by the FDA

Informed consent requires that each potential subject must be adequately informed of the potential risks of the study and the discomfort it may entail.(38) However, Oxitec has misinformed the residents of the Keys and California about the potential risks and discomfort involved in this study.

Further, misleading claims are made in the survey such as "to control the species of mosquitoes that spreads dengue fever". This implies that this trial will control dengue, yet there is no dengue, nor was there any dengue in the Keys at the time of the survey. Oxitec makes further misleading claims such as *"Releases of Oxitec mosquitoes over a sustained period of time can safely reduce the mosquito population and therefore the incidence of dengue fever."*(79) Oxitec claims, *"pilot projects conducted last year and high levels of support for them demonstrated the value of Friendly™ Aedes aegypti technology for communities in our country that are working to combat the growing threat of dengue."*(193) Oxitec has also made misleading claims which leads to articles titled, "FDA says GMO mosquito likely OK to fight Zika in Florida" Oxitec's Haydn Parry is quoted as saying, *"I'm sure there will be some that don't agree. But we have a very significant public health threat before us. Time is not on our side if you look at how Zika has been spreading. The sooner we can get going and show what we can do, the sooner we can make a difference in the fight against this virus."*(75) Even though there are no cases of Zika in the Florida Keys and none of the objectives for this trial include reducing Zika. Oxitec admitted during a town hall meeting in the Keys that, *"In terms of dengue transmission we have done 4 or 5 trials now, but those trials have been too small to be able to show any sort of effect on dengue."*(37) Since this trial is as small

as other Oxitec trials there is no potential reduction in any mosquito-borne diseases either. Therefore, Oxitec has misinformed the residents of the Keys and California about the anticipated benefits and the potential risks and discomfort involved in this study.

In a poll that *"was paid for by Oxitec"*(88) they say to those surveyed, *"The U.S. Food & Drug Administration (FDA) has released a preliminary finding that this type of genetically engineered mosquito will NOT have a negative impact on public health or the environment"*(89) What the FDA actually said is, *"FDA found that the probability of adverse impacts on human or other animal health is negligible or low."* Claiming, *"will NOT have a negative impact"* is very different from *"negligible or low."* Claiming *"will NOT"* is claiming that the FDA said nothing can possibly go wrong, it is a mockery of the regulatory process and the FDA. It is Oxitec's duty and responsibility to adequately inform each person of *"the anticipated benefits and potential risks of the study and the discomfort it may entail"* and *"all inconveniences and hazards reasonably to be expected; and the effects upon his health or person, which may possibly come from his participation in the experiment."* Oxitec not only failed to do this, but they misled people about the risks.

Due to the misinformation given to most Keys residents, Oxitec must refrain from further spreading misinformation and ensure that each resident is properly informed that since this trial is as small as other Oxitec trials there is no potential reduction in any mosquito-borne diseases. So, there is no likelihood that the populations in which the research is carried out stand to benefit from the results of the research. Oxitec must also ensure that each resident is properly informed of the potential risks of the study and the discomfort it may entail. The EPA cannot remain silent on incorrect information from Oxitec. Oxitec cannot be allowed to put any story in the media without any challenge or fact check as they have done in the past. This misinformation spread by Oxitec is not only anti-science, it also undermines the regulatory process. The FDA states, *"a disease claim, that is, a claim to diagnose, cure, mitigate, treat, or prevent disease. Disease claims require prior approval by FDA and may be made only for products that are approved drug products or for foods under separate legal provisions that apply to claims called "health claims." "First, the law says you can make these claims if you have substantiation that the claims are truthful and not misleading. You must have this substantiation before you make the claims. Second, you must notify the FDA that you are using the claim within 30 days of first marketing your product. Third, the claim must include a mandatory disclaimer statement that is provided for in the law."*(80)

Oxitec has clearly made a disease claim as is defined by the FDA. However, it appears they have not followed the law in doing so. Therefore, the EPA must urge the FDA to investigate Oxitec for violation of the law and for the FDA to require approval of these genetically modified mosquitoes as Oxitec is clearly making claims of disease reduction which is outside of the EPA's jurisdiction.

4. Medical Risk

Oxitec must conduct allergen tests of every person in the trial area to ensure they are not allergic reactions before releasing the millions of genetically engineered mosquitoes they plan to release. If someone in the test area is allergic this could cause a severe allergic reaction and a medical emergency. Oxitec cannot take risks with the health of the people in the trial area and all potential medical risks

must be considered, including but not limited to testing every individual in the trial area for allergenicity potential.

5. Post-trial monitoring

Aedes aegypti eggs may stay viable and resistant to desiccation for up to 450 days(34). There is no mention of post-trial monitoring of potentially viable eggs for this duration after the trial.

6. Liability

Oxitec makes no mention on how they intend to deal with the issue of liability. Florida Keys are not simply an island location. They are connected via roadway to the state of Florida, which is then connected via roadway to the rest of the continental U.S. and other countries. So for the question of "Will the GE mosquitoes travel outside of the Keys?" The answer is an almost definite "Yes". Some studies observed *Aedes aegypti* traveling up to 800 meters(59) as much as 1000 meters across water and up to 2,500 meters in some cases(96). This distance could easily place a genetically engineered mosquito in a vehicle intended for another state or another country. Mosquitoes are believed to frequently travel long distances via boat, automobile, etc.(100) and this is also likely true for Florida.(101). It is believed that the recent presence of *Aedes aegypti* in California was caused by commerce via air, railroad, or trucks traveling from the southern U.S.(102). Even *Aedes aegypti* found as far away as the Netherlands are believed to have traveled there in airplane tires arriving from southern Florida.(103,104) With over 3 million visitors to the Florida Keys(25) a year, and numerous vehicles traveling in and out of the area, these genetically engineered mosquitoes escaping the test area is an extremely likely scenario. Since there is near certainty that a GE mosquito will travel outside of the trial area, and perhaps into another county, state or country, Oxitec must have a funds set aside to deal with lawsuits that may occur when individuals, counties, states or countries, who did not agree to this experiment, file lawsuits as a result.

If the earlier mutation estimates are correct, OX5034 female mosquitoes may be present at some point in the Keys and California. What happens when people have been bitten? It is still possible that these mosquitoes do have these proteins in their saliva. Also, there is a risk of an allergic response in residents exposed to the GE mosquitoes. Oxitec must therefore put aside funds to deal with the possibility of lawsuits in the case of such a scenario. It should also be mandatory that Oxitec is insured in the case that these, or other, events occur. These details are also missing from the documentation.

Since Oxitec admits, *"Uncertainty can be reduced by obtaining or generating more data on particular aspects"*(121) they should have no problem conducting the suggested studies to generate more data and reduce uncertainty before releasing these GE mosquitoes into the environment in the Florida Keys.

EPA must reject Oxitec's application for genetically engineered mosquitoes and:

- Complete a full environmental impact statement on Oxitec's GMO mosquito release proposal.

- Have a committee of independent ecologists and entomologists, public health experts (including dengue fever and zika virus specialists), and other key experts and public stakeholders review the proposal from Oxitec.

- Convene a public meeting in the Florida Keys, advertised in the Federal Register for the review of the company's proposal with the above committee present.

Scientists have significant concerns about how GE mosquitoes could impact the health of people and of critical ecosystems. Once released into the environment, this new, living engineered organism cannot be "recalled"; GE mosquitoes could reproduce and cause unintended changes in the ecosystem.

- As a first step, the EPA should clarify the legal basis under which it proposes that Oxitec should be released from the contained use requirements of its import permit, in order to allow its GE insects to be deliberately released into the environment.

- A full EIS should be prepared under the National Environmental Policy Act (NEPA), and this should be subject to further consultation. The EIS should include consideration of the EPA's responsibilities under other environmental legislation, including the Endangered Species Act.

- Although further demonstration of efficacy would be necessary before Oxitec could submit an application to register a pesticide under section 136a of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), more laboratory and caged trials are first essential to establish that use of the pesticide under the permit, and its method of delivery via living genetically engineered (GE) pest organisms, does not cause unreasonable adverse effects on the environment.

Our government agencies must not rely only on data from companies that would profit from genetically engineered organisms to decide what information the public and regulators should know. Until the above requests have been met, the application for the field release of genetically engineered mosquitoes must not be allowed to move forward.__

References :

1. Skinner, M. K. (2014). Environmental stress and epigenetic transgenerational inheritance. BMC medicine, 12(1), 1. http://web.archive.org/web/20160407000656/http://bmcmedicine.biomedcentral.com/articles/10.1186/s12916-014-0153-y

2. Ricarda A. Steinbrecher (2010) Scientific opinion to the Department of Biosafety, Ministery of Natural Resources and Environment of Malaysia http://web.archive.org/web/20160319055852/http://www.econexus.info/publication/release-gm-mosquito-aedes-aegypti-ox513a

3. U.S. Department of Health & Human Services (2005) The Nuremberg Code http://web.archive.org/web/20160402051557/http://www.hhs.gov/ohrp/archive/nurcode.html

4. Prof. Joe Cummins and Dr. Mae-Wan Ho (2008) Terminator Mosquitoes to Control Dengue? http://web.archive.org/web/20160402055220/http://www.i-sis.org.uk/Terminator_Mosquitoes_to_Control_Dengue.php

5. Dr. Mae-Wan Ho (2001) Terminator insects give wings to genome invaders http://web.archive.org/web/20160320102745/http://www.i-sis.org.uk/terminsects-pr.php

6. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology 22 – 25 January 2001 http://web.archive.org/web/20030328162631/http://www.fao.org/es/ESN/food/pdf/allergygm.pdf

7. US Public Overwhelmingly Supports Use of GMO Mosquitoes to Help Control Zika Virus http://web.archive.org/web/20160407002152/https://extension.purdue.edu/Pages/article.aspx?intItemID=14074

8. Oxitec Ltd http://web.archive.org/web/20160324023843/https://twitter.com/Oxitec/status/711982182638690304

9. Townhall Meeting 3/15/12 https://www.youtube.com/watch?v=5WQkM-yc7QI

10. STEPHANIE NOLEN (2016) WHO may be leading Brazil down wrong path on Zika virus http://web.archive.org/web/20160325031221/http://www.theglobeandmail.com/news/world/who-may-be-leading-brazil-down-wrong-path-on-zika-virus/article29390468/

11. Michael D. Cobb (2013) Public Perceptions of Mosquito Control Efforts in the Florida Keys: A Survey of Florida Keys Residents, January 2013 http://web.archive.org/web/20160407002701/http://keysmosquito.org/wp-content/uploads/2013/02/All-Keys-Survey-Results-2013.pdf

12. Genetically modified mosquitoes' survival rate concealed http://web.archive.org/web/20160319003736/http://www.foe.org/news/news-releases/2012-01-genetically-modified-mosquitoes-survival-rate

13. EDUARDO B BESERRA, CARLOS R M FERNANDES, JOSÉ T DESOUSA, ERALDO M DE FREITAS, KELIANA D SANTOS (2010) The Effect of Water Quality in the Life Cycle and in the Attraction for the Egg Oviposition of Aedes aegypti (L.) (Diptera: Culicidae). Neotropical Entomology 39(6):1016-1023 "It can be concluded that A. aegypti does not demonstrate any particular preference to lay eggs on clean water." http://www.scielo.br/pdf/ne/v39n6/v39n6a26.pdf

14. LAWRENCE J. HRIBAR, JOSHUA J. VLACH, DAVID J. DEMAY, SHANNON S. JAMES JENNIFER S. FAHEY AND EDSEL M. FUSSELL (2004) MOSQUITO LARVAE (CULICIDAE) AND OTHER DIPTERA ASSOCIATED WITH CONTAINERS, STORM DRAINS, AND SEWAGE TREATMENT PLANTS IN THE FLORIDA KEYS, MONROE COUNTY, FLORIDA "Sewage treatment plants, septic tanks, and cesspits were larval development sites for Cx. quinquefasciatus, Cx. nigripalpus, and Ae. aegypti." http://web.archive.org/web/20101206000141/http://www.fcla.edu/FlaEnt/fe87p199.pdf

15. Barrera R, Amador M, Diaz A, Smith J, Munoz-Jordan JL, Rosario Y. (2008) Unusual productivity of *Aedes aegypti* in septic tanks and its implications for dengue control. Med Vet Entomol. 2008 "Geographical Information Systems identified significant clustering of adult mosquitoes, which led to the discovery of underground aquatic habitats (septic tanks) that were producing large numbers of *Ae.*

aegypti and *Culex quinquefasciatus (Say)* in the treated town. We calculated that septic tanks could produce > 18 000 Ae. aegypti and approximately 170 000 *Cx quinquefasciatus* adults per day. Septic tanks are likely to be common and widespread in suburban and rural Puerto Rico, where, apparently, they can contribute significantly to the maintenance of island-wide dengue virus endemicity." http://www.ncbi.nlm.nih.gov/pubmed/18380655

16. Dengue and the *Aedes aegypti* mosquito. Dengue Branch, San Juan, PR "They lay eggs during the day in water containing organic material (e.g., decaying leaves, algae, etc.) in containers with wide openings and prefer dark-colored containers located in the shade." http://web.archive.org/web/20160404035221/http://www.cdc.gov/dengue/resources/30Jan2012/aegyptifactsheet.pdf

17. Santana AL, Roque RA, Eiras AE (2006) Characteristics of grass infusions as oviposition attractants to Aedes (Stegomyia) (Diptera:Culicidae). J Med Entomol. 43(2):214-20 "Ovitraps baited with infusions of Panicum maximum (Jacq.) collected significantly more eggs than controls containing tap water." http://www.ncbi.nlm.nih.gov/pubmed/16619601

18. Eloína Santos; Juliana Correia; Luciana Muniz; Marcos Meiado; Cleide Albuquerque (2010) Oviposition activity of Aedes aegypti L. (Diptera: Culicidae) in response to different organic infusions. Neotrop. entomol. vol.39 no.2 Londrina "The two types of infusion tested achieved a significantly higher oviposition response than the distilled water" http://www.scielo.br/pdf/ne/v39n2/v39n2a23.pdf

19. McPhatter, Lee P. Olsen, Cara H. Debboun, Mustapha (2009) Comparison of the attractiveness of organic infusions to the standard CDC gravid mosquito trap. U.S.Army Medical Department Journal "Aedes aegypti (L) females were attracted to horse manure infusions" http://web.archive.org/web/20160407003548/http://www.biomedsearch.com/article/Comparison-attractiveness-organic-infusions-to/242963594.html

20. G. Hamscher, S. Sczesny, H. Nau, Tetracycline and Chlortetracycline Residues in Soil Fertilized with Liquid Manure "In a screening of 62 pig slurry samples 9 were found positive for tetracycline with amounts of 5 to 24 mg/L." "Finally we conclude, that our studies show that etracyclines, which are frequently used worldwide, are not only persistent inanimal slurry but also in soil in significant amounts." "A recently published study of Germanys Federal Environmental Agency showed, that the degradation rate of tetracycline in liquid manure is approximately 50 % in 5 months." http://web.archive.org/web/20150516010514/http://agriculture.de/acms1/conf6/ws4tetra.htm

21. Liu H, Zhang G, Liu CQ, Li L, Xiang M. (2009) The occurrence of chloramphenicol and tetracyclines in municipal sewage and theNanming River, Guiyang City, China. J Environ Monit. 11(6):1199-205. "The occurrence of antibiotics including chloramphenciol (CAP), oxytetracycline (OTC) and tetracycline (TC) was studied in municipal sewage.""In municipal sewage, CAP, OTC and TC concentrations were in the range of 5.8-47.4, 0.16-5.7 and 0.7-65.2 microg L(-1), respectively." http://www.ncbi.nlm.nih.gov/pubmed/19513451

22. S. E. Mason, R. E. Baynes, G. W. Almond, J. E. Riviere and A. B. Scheidt (2009) Pharmacology of tetracycline water medication in swine. J ANIM SCI vol. 87 no. 10 3179-3186 "Twenty-four barrows, divided into 1 control group (of nontreated animals) and 3 equally sized treatments groups (n = 6/group),

were treated with tetracycline water medication for 5 d at 125, 250, and 500 mg/L."
http://web.archive.org/web/20120622173106/http://jas.fass.org/content/87/10/3179.full

23. Dengue and the Aedes aegypti mosquito. Dengue Branch, San Juan, PR "They lay eggs during the day in water containing organic material (e.g., decaying leaves, algae, etc.) in containers with wide openings and prefer dark-colored containers located in the shade."
http://www.cdc.gov/dengue/resources/30Jan2012/aegyptifactsheet.pdf

24. Woodbridge A. Foster, Arden O. Lea (1975) Renewable fecundity of male Aedes aegypti following replenishment of seminal vesicles and accessory glands Journal of Insect Physiology Volume 21, Issue 5, May, Pages 1085–1090 http://www.sciencedirect.com/science/article/pii/0022191075901201

25. Frink, Stephen Diving America's Historical Island Paradise "A study conducted between June 1995 and May of 1996 by the National Oceanic and Atmospheric Administration (NOAA) concluded that just over 3 million tourists came to the Florida Keys each year."
http://web.archive.org/web/20160401043040/http://www.fla-keys.com/diving/divingparadise.cfm

26. Michael D. Cobb (2013) Public Perceptions of GE Mosquito Control Efforts in Key West: An In-Person Survey of 205 K.W. Residents, January 1-5, 2013
http://web.archive.org/web/20160407004212/http://keysmosquito.org/wp-content/uploads/2013/02/Key-West-Survey-Results-2013.pdf

27. Tu WC, Chen CC, Hou RF. (1998) Ultrastructural studies on the reproductive system of male Aedes aegypti (Diptera: Culicidae) infected with dengue 2 virus. J Med Entomol. Jan;35(1):71-6.
http://www.ncbi.nlm.nih.gov/pubmed/9542348

28. Mavale M, Parashar D, Sudeep A, Gokhale M, Ghodke Y, Geevarghese G, Arankalle V, Mishra AC. (2010) Venereal transmission of chikungunya virus by Aedes aegypti mosquitoes (Diptera: Culicidae). Am J Trop Med Hyg. 83(6):1242-4. "Experiments were conducted to demonstrate the role of male Aedes aegypti mosquitoes in the maintenance and transmission of chikungunya virus (CHIKV) to female mosquitoes. We demonstrated that infected male mosquitoes are capable of infecting females during mating." http://web.archive.org/web/20160407004938/http://www.ajtmh.org/content/83/6/1242.full

29. Scott, T. W., Takken, W., Knols, B. G., & Boete, C. (2002). The ecology of genetically modified mosquitoes. Science, 298(5591), 117-119. "effectively transmit dengue virus even at very low population densities because they preferentially and frequently bite humans."

30. Canyon DV, Hii JL, Muller R. (1999) Effect of diet on biting, oviposition, and survival of Aedes aegypti (Diptera: Culicidae). J Med Entomol 36(3):301-8. "biting occurred opportunistically without regard for previously observed crepuscular or diurnal rhythms." http://www.ncbi.nlm.nih.gov/pubmed/10337099

31. CanyonDV, Hii JLK and Muller R. (1998) Multiple host feeding behaviour in Aedes aegypti. Annals of Tropical Medicine and Parasitology 92:311-316. "Multiple host-feeding is a more important factor than multiple blood- meals (as the latter may be taken from the same host), with a greater impact on the dynamics of vector-borne diseases."
http://manoa-hawaii.academia.edu/DeonCanyon/Papers/1034627/98_Canyon_-_Multiple_host_feeding_behaviour_in_Aedes_aegypti

32. Hobbs JH, Hughes EA, Eichold BH 2nd. (1991) Replacement of Aedes aegypti by Aedes albopictus in Mobile, Alabama. J Am Mosq Control Assoc. Sep;7(3):488-9. http://www.ncbi.nlm.nih.gov/pubmed/1791461

33. GM Aedes aegypti Research http://webcache.googleusercontent.com/search?q=cache:http://www.imr.gov.my/highlights-featured-articles/1119-gm-aedes-aegypti-research-v2.html

34. Rodrigo Braga Moruzzi, Ademir Pacelli Barbassa, Thaís Helena Prado Corrêa, Lorena Avelina Rojas Gutierrez, Bernardo Arantes do Nascimento Teixeira (2014) Aedes Aegypti Proliferation in Stormwater Exposed in Best Management Practices. International Journal of Civil & Environmental Engineering IJCEE-IJENS Vol:14 No:02 http://web.archive.org/web/20150315111249/http://ijens.org/Vol_14_I_02/137206-1402-8989-IJCEE-IJENS.pdf

35. JOHN DeSANTIS (2012) Fear underlies mosquito proposal July 21 http://web.archive.org/web/20151003223437/http://keysnews.com/node/41181

36. JULIE BUTLER (2014) Oxitec Still Pursuing Trial Release of GM Olive Flies in Spain http://web.archive.org/web/20150205062034/http://www.oliveoiltimes.com/olive-oil-making-and-milling/oxitec-still-pursuing-release-gmo-olive-flies-spain/38079

37. Mosquito Control Board Town Hall Meeting https://www.youtube.com/watch?v=AQOSRg3l0Ys

38.WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI Ethical Principles for Medical Research Involving Human Subjects http://web.archive.org/web/20150724102003/http://www.fda.gov/ohrms/dockets/dockets/06d0331/06D-0331-EC20-Attach-1.pdf

39. Salazar, M. I., Richardson, J. H., Sánchez-Vargas, I., Olson, K. E., & Beaty, B. J. (2007). Dengue virus type 2: replication and tropisms in orally infected Aedes aegypti mosquitoes. BMC microbiology, 7(1), 9. http://web.archive.org/web/20160105194010/http://bmcmicrobiol.biomedcentral.com/articles/10.1186/1471-2180-7-9

40.Linda Qiu (2016) No evidence to support rumors tying Zika to genetically modified mosquitoes http://web.archive.org/web/20160329172527/http://www.politifact.com/global-news/statements/2016/feb/09/viral-image/no-evidence-support-rumors-tying-zika-genetically-/

41. Dubrulle M, Mousson L, Moutailler S, Vazeille M, Failloux A-B (2009) Chikungunya Virus and Aedes Mosquitoes: Saliva Is Infectious as soon as Two Days after Oral Infection. PLoS ONE 4(6): e5895. doi:10.1371/journal.pone.0005895

42. Diagne, C. T., Faye, O., Guerbois, M., Knight, R., Diallo, D., Faye, O., ... & Diallo, M. (2014). Vector competence of Aedes aegypti and Aedes vittatus (diptera: Culicidae) from Senegal and Cape Verde archipelago for West African lineages of chikungunya virus. The American journal of tropical medicine and hygiene, 91(3), 635-641. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4155570/

43. RENÉE ALEXANDER (2014) Engineering Mosquitoes to Spread Health http://web.archive.org/web/20160312135426/http://www.theatlantic.com/health/archive/2014/09/engineering-mosquitoes-to-stop-disease/379247/

44. Paupy C, Ollomo B, Kamgang B, Moutailler S, Rousset D, Demanou M, et al. Comparative Role of Aedes albopictus and Aedes aegypti in the Emergence of Dengue and Chikungunya in Central Africa. Vector Borne Zoonotic Dis. 2009 http://web.archive.org/web/20160407010356/http://www.mivegec.ird.fr/images/stories/PDF_files/0908.pdf

45. Thavara, U., Tawatsin, A., & Nagao, Y. (2014). Simulations to compare efficacies of tetravalent dengue vaccines and mosquito vector control. Epidemiology & Infection, 142(06), 1245-1258. http://web.archive.org/web/20160407010453/http://journals.cambridge.org/download.php?file=%2FHYG%2FHYG142_06%2FS0950268813001866a.pdf&code=da745af28cc76cc7ad870197ef071dd8

46. Diagne, Cheikh T., Diawo Diallo, Oumar Faye, Yamar Ba, Ousmane Faye, Alioune Gaye, Ibrahima Dia, Scott C. Weaver, Amadou A. Sall, and Mawlouth Diallo. "Potential of selected Senegalese Aedes spp. mosquitoes (Diptera: Culicidae) to transmit Zika virus." BMC infectious diseases 15, no. 1 (2015): 492. http://web.archive.org/web/20160128202115/http://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-015-1231-2

47. Mudrak, TP, and J. F. Zhukov. "The potential of horizontal gene transfer of GMOs in the meat and milk of domestic and wild animals." Food resources. Series: Engineering 3 (2014): 135-145. http://www.irbis-nbuv.gov.ua/cgi-bin/irbis_nbuv/cgiirbis_64.exe?C21COM=2&I21DBN=UJRN&P21DBN=UJRN&IMAGE_FILE_DOWNLOAD=1&Image_file_name=PDF/prrteh_2014_3_27.pdf

48. Tudisco, Raffaella, Serena Calabrò, Monica Isabella Cutrignelli, Giuseppe Moniello, Micaela Grossi, Vincenzo Mastellone, Pietro Lombardi, Maria Elena Pero, and Federico Infascelli. "Genetically modified soybean in a goat diet: Influence on kid performance." Small Ruminant Research 126 (2015): 67-74. http://www.smallruminantresearch.com/article/S0921-4488(15)00052-8/fulltext

49. Ran, T., Mei, L., Lei, W., Aihua, L., Ru, H. and Jie, S. (2009), Detection of transgenic DNA in tilapias (Oreochromis niloticus, GIFT strain) fed genetically modified soybeans (Roundup Ready). Aquaculture Research, 40: 1350–1357. doi: 10.1111/j.1365-2109.2009.02187.x http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2109.2009.02187.x/full

50. Tudisco, R., V. Mastellone, M. I. Cutrignelli, P. Lombardi, F. Bovera, N. Mirabella, G. Piccolo, S. Calabro, L. Avallone, and F. Infascelli. "Fate of transgenic DNA and evaluation of metabolic effects in goats fed genetically modified soybean and in their offsprings." Animal 4, no. 10 (2010): 1662-1671. http://journals.cambridge.org/action/displayFulltext?type=6&fid=7875680&jid=ANM&volumeId=4&issueId=10&aid=7875679&bodyId=&membershipNumber=&societyETOCSession=&fulltextType=RN&fileId=S1751731110000728

51. CHAINARK, P., SATOH, S., HINO, T., KIRON, V., HIRONO, I. and AOKI, T. (2006), Availability of genetically modified soybean meal in rainbow trout Oncorhynchus mykiss diets. Fisheries Science, 72: 1072–1078. doi: 10.1111/j.1444-2906.2006.01258.x

52. Sustainable Pulse Dec 9 2013 Oxitec Cancels GM Flies Release after Government Health Questions http://web.archive.org/web/20150928081937/http://sustainablepulse.com/2013/12/09/oxitec-cancels-gm-flies-release-government-health-questions/

53. Lawrence J. Hribar, Andrea L. Leal , David J. DeMay and Adriane N. Tambasco (2011) Mosquitoes (Insecta: Diptera: Culicidae) of the Florida Keys, Florida, United States of America http://web.archive.org/web/20160407012028/http://www.checklist.org.br/getpdf?SL071-10

54. Dr. Michael Cobb, Dr. Fred Gould (2012) NCSU, Genetically Engineered Mosquito Survey http://web.archive.org/web/20160321173429/https://news.ncsu.edu/2012/08/wms-cobb-mosquitoes/

55. Mila de Mier (2012) Say No to Genetically Modified Mosquitoes Release in the Florida Keys http://www.change.org/petitions/say-no-to-genetically-modified-mosquitoes-release-in-the-florida-keys

56. Chung Youne Kow, Lim Loo Koon, and Pang Fung Yin (2001) Detection of Dengue Viruses in Field Caught Male Aedes aegypti and Aedes albopictus (Diptera: Culicidae) in Singapore by Type-Specific PCR Journal of Medical Entomology 38(4):475-479. http://www.bioone.org/doi/abs/10.1603/0022-2585-38.4.475?journalCode=ment

57. Aedes aegypti Environmental Assessment. http://www.genewatch.org/uploads/f03c6d66a9b354535738483c1c3d49e4/Environmental_Assessment.pdf

58. H.L. Lee and A. Rohani (2005) Transovarial Transmission of Dengue Virus in Aedes aegypti and Aedes albopictus in Relation to Dengue Outbreak in an Urban Area in Malaysia Dengue Bulletin - Volume 29 http://repository.searo.who.int/bitstream/123456789/16018/2/dbv29p106.pdf

59. Maciel-de-Freitas R1, Lourenço-de-Oliveira R. (2009) Presumed unconstrained dispersal of Aedes aegypti in the city of Rio de Janeiro, Brazil. Rev Saude Publica. Feb;43(1):8-12. http://www.ncbi.nlm.nih.gov/pubmed/19169571

60. CHAINARK, P., SATOH, S., HIRONO, I., AOKI, T. and ENDO, M. (2008), Availability of genetically modified feed ingredient: investigations of ingested foreign DNA in rainbow trout Oncorhynchus mykiss. Fisheries Science, 74: 380–390. doi: 10.1111/j.1444-2906.2008.01535.x

61. Hanaa A. S. Oraby, Mahrousa M. H. Kandil, Amal A. M. Hassan, Hayam A. Al-Sharawi (2014) Addressing the issue of horizontal gene transfer from a diet containing genetically modified components into rat tissues. Afr. J. Biotechnol. Vol. 13 No. 48 http://www.academicjournals.org/journal/AJB/article-full-text/BE5331948800

62. Einspanier, R., Lutz, B., Rief, S., Berezina, O., Zverlov, V., Schwarz, W., & Mayer, J. (2004). Tracing residual recombinant feed molecules during digestion and rumen bacterial diversity in cattle fed transgene maize. European Food Research and Technology, 218(3), 269-273. http://physio.wzw.tum.de/fileadmin/DNA_und_dem_Cry1Ab-Protein/Reprints/Einspanier-et-al-2004.pdf

63. Martın-Orúe, S. M., O'Donnell, A. G., Ariño, J., Netherwood, T., Gilbert, H. J., & Mathers, J. C. (2002). Degradation of transgenic DNA from genetically modified soya and maize in human intestinal simulations. British Journal of Nutrition, 87, 533-542.

https://www.researchgate.net/publication/11308007_Degradation_of_transgenic_DNA_from_genetically_modified_soya_and_maize_in_human_intestinal_simulations

64. Genetically Modified InsectFactories:A New Source of Superbugs? February 2015 http://www.genewatch.org/uploads/f03c6d66a9b354535738483c1c3d49e4/Antibiotic_GWbrief_fin.pdf

65. Miller MJ, Loaiza JR (2015) Geographic Expansion of the Invasive MosquitoAedes albopictus across Panama—Implications for Control of Dengue and Chikungunya Viruses. PLoS Negl Trop Dis 9(1): e0003383. doi:10.1371/journal.pntd.0003383

66. Natural Health News, April 21, 2015, Contrary to Mosquito Control Surveys, 75% of Key Haven Residents Oppose GM Mosquitoes : http://web.archive.org/web/20150628092552/http://www.naturalblaze.com/2015/04/contrary-to-mosquito-control-surveys-75.html

67. Massonnet-Bruneel, Blandine, et al. "Fitness of transgenic mosquito Aedes aegypti males carrying a dominant lethal genetic system." (2013): e62711. http://web.archive.org/web/20160329051120/http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0062711

68. Duggan, P. S., Chambers, P. A., Heritage, J., & Forbes, J. M. (2003). Fate of genetically modified maize DNA in the oral cavity and rumen of sheep. British Journal of Nutrition, 89(02), 159-166. https://www.researchgate.net/publication/10910019_Fate_of_genetically_modified_maize_DNA_in_the_oral_cavity_and_rumen_of_sheep

69. Robinson AS, Franz G, Atkinson PW (2004) Insect transgenesis and its potential role in agriculture and human health. Insect Biochemistry and Molecular Biology, 34(2), 113-120. https://www.researchgate.net/publication/8778146_Insect_transgenesis_and_its_potential_role_in_agriculture

70. Alphey N, Bonsall B, Alphey A (2011) Modeling resistance to genetic control of insects. Journal of Theoretical Biology, 270, 42-55 http://www.ncbi.nlm.nih.gov/pubmed/21075122

71. Whitsett JA, Perl A-KT. Conditional Control of Gene Expression in the Respiratory Epithelium: A Cautionary Note. American Journal of Respiratory Cell and Molecular Biology. 34(5):519–520. http://www.atsjournals.org/doi/pdf/10.1165/rcmb.F310

72. Han HJ, Allen CC, Buchovecky CM, et al. (2012) Strain background influences neurotoxicity and behavioral abnormalities in mice expressing the tetracycline transactivator. J Neurosci. 32(31):10574–10586. doi:10.1523/JNEUROSCI.0893-12.2012.

73. Andrew Lima, Diane D. Lovin, Paul V. Hickner, and David W. Severson (2015) Evidence for an Overwintering Population of Aedes aegypti in Capitol Hill Neighborhood, Washington, DC. Am J Trop Med Hyg 2016 94:231-235; November 2, 2015, doi:10.4269/ajtmh.15-0351

74. Netherwood, T., Martín-Orúe, S. M., O'Donnell, A. G., Gockling, S., Graham, J., Mathers, J. C., & Gilbert, H. J. (2004). Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. Nature biotechnology, 22(2), 204-209. http://www.nature.com/nbt/journal/v22/n2/abs/nbt934.html

75. Sandee LaMotte (2016) FDA says GMO mosquito likely OK to fight Zika in Florida http://web.archive.org/web/20160405125713/http://www.cnn.com/2016/03/11/health/zika-florida-gmo-mosquito-fda-assessment/index.html

76. Damian Pattinson (2012) PLOS ONE Launches Reproducibility Initiative http://web.archive.org/web/20160322023211/http://blogs.plos.org/everyone/2012/08/14/plos-one-launches-reproducibility-initiative/

77. PRESS RELEASE: Oxitec and MRCU report 80% suppression of a dengue mosquito population in Grand Cayman by release of engineered sterile male mosquitoes – Nature Biotechnology http://web.archive.org/web/20160320224018/http://www.oxitec.com/press-release-oxitec-and-mrcu-report-80-suppression-of-a-dengue-mosquito-population-in-grand-cayman-by-release-of-engineered-sterile-male-mosquitoes-nature-biotechnology/

78. TRIALLING A NEW WAY TO FIGHT MOSQUITOS IN THE FLORIDA KEYS http://web.archive.org/web/20160407120041/http://www.oxitec.com/wpcms/wp-content/uploads/Information-about-Florida-trial-EA-2015.pdf?db0f11

79. TRIALLING A NEW WAY TO FIGHT MOSQUITOS IN THE FLORIDA KEYS http://web.archive.org/web/20150922031715/http://www.oxitec.com/news-and-views/topic-pages-safety-and-sustainability/ridl-sit-and-dengue-fever/

80. U.S. Food and Drug Administration (2002) Guidance for Industry: Structure/Function Claims, Small Entity Compliance Guide http://web.archive.org/web/20160319062841/http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/DietarySupplements/ucm103340.htm

81. Peng, Z., Beckett, A. N., Engler, R. J., Hoffman, D. R., Ott, N. L., & Simons, F. E. R. (2004). Immune responses to mosquito saliva in 14 individuals with acute systemic allergic reactions to mosquito bites. Journal of Allergy and Clinical Immunology, 114(5), 1189-1194. http://www.jacionline.org/article/S0091-6749(04)02218-3/fulltext

82. McCormack, D. R., Salata, K. F., Hershey, J. N., Carpenter, G. B., & Engler, R. J. (1995). Mosquito bite anaphylaxis: immunotherapy with whole body extracts. Annals of allergy, asthma & immunology: official publication of the American College of Allergy, Asthma, & Immunology, 74(1), 39-44. http://www.ncbi.nlm.nih.gov/pubmed/7719881

83. Braks, M. A., Honório, N. A., Lourenço-De-Oliveira, R., Juliano, S. A., & Lounibos, L. P. (2003). Convergent habitat segregation of Aedes aegypti and Aedes albopictus (Diptera: Culicidae) in southeastern Brazil and Florida. Journal of Medical Entomology, 40(6), 785-794. https://about.illinoisstate.edu/sajulian/Documents/publications/braks%20et%20al%2003.pdf

84. Peng, Z., Ho, M. K., Li, C., & Simons, F. E. R. (2004). Evidence for natural desensitization to mosquito salivary allergens: mosquito saliva specific IgE and IgG levels in children. Annals of Allergy, Asthma & Immunology, 93(6), 553-556. http://www.ncbi.nlm.nih.gov/pubmed/15609764

85. Lam, P. H., Boon, C. S., Yng, N. Y., & Benjamin, S. (2010). Aedes albopictus control with spray application of Bacillus thuringiensis israelensis, strain AM 65-52. Southeast Asian Journal of Tropical Medicine and Public Health, 41(5), 1071. http://www.ncbi.nlm.nih.gov/pubmed/21073027

86. Florida Keys Mosquito Control District Chemical Labels and MSDS
http://web.archive.org/web/20160323223637/http://keysmosquito.org/labels/

87. Oxitec Ltd. April 10, 2014 Dengue Mosquito Population Reduced by 79% in Jacobina, Bahia, Using Oxitec Solution
http://web.archive.org/web/20140419163339/http://www.prnewswire.com/news-releases/dengue-mosquito-population-reduced-by-79-in-jacobina-bahia-using-oxitec-solution-254717291.html

88. Marc Caputo 04/13/16 Poll: strong support in Florida, nation for Zika-fighting genetically modified mosquito test
http://web.archive.org/web/20160416190028/http://www.politico.com/tipsheets/florida-playbook/2016/04/jacksonville-cabal-mulls-rep-crenshaw-replacement-poll-big-support-for-genetically-modified-skeeters-in-zika-fight-whats-rubio-up-to-steven-spielberg-backs-murphy-gender-queer-quiz-leads-to-suspension-213722

89. Mosquito Omnibus Survey Results United States Results Field Dates: 1 - 4 April 2016
http://web.archive.org/web/20160416190506/http://www.politico.com/f/?id=00000154-0cc7-d611-a7fe-9fc72fe00000

90. Centers for Disease Control and Prevention Zika Virus
http://web.archive.org/web/20160408133645/http://www.cdc.gov/zika/transmission/

91. Food and Agriculture Organization of the United Nations Zika Virus
http://web.archive.org/web/20160411075143/http://www.fao.org/zika-virus/en/

92. De Liguoro, M., Cibin, V., Capolongo, F., Halling-Sørensen, B., & Montesissa, C. (2003). Use of oxytetracycline and tylosin in intensive calf farming: evaluation of transfer to manure and soil. Chemosphere, 52(1), 203-212. http://www.sciencedirect.com/science/article/pii/S0045653503002844

93. Farjana, T., & Tuno, N. (2013). Multiple blood feeding and host-seeking behavior in Aedes aegypti and Aedes albopictus (Diptera: Culicidae). Journal of medical entomology, 50(4), 838.
https://www.researchgate.net/profile/Nobuko_Tuno/publication/255713319_Multiple_blood_feeding_and_host-seeking_behavior_in_Aedes_aegypti_and_Aedes_albopictus_(Diptera_Culicidae)/links/00b4951f0bae99c471000000.pdf

94. Lee, J. J., & Klowden, M. J. (1999). A male accessory gland protein that modulates female mosquito (Diptera: Culicidae) host-seeking behavior. Journal of the American Mosquito Control Association-Mosquito News, 15(1), 4-7.

95. Liles, J. N., & Delong, D. M. (1960). The longevity and productivity of adult male and female Aedes aegypti when reared separately and together on three different diets. Annals of the Entomological Society of America, 53(2), 277-280. http://aesa.oxfordjournals.org/content/53/2/277

96. Honório, N. A., Silva, W. D. C., Leite, P. J., Gonçalves, J. M., Lounibos, L. P., & Lourenço-de-Oliveira, R. (2003). Dispersal of Aedes aegypti and Aedes albopictus (Diptera: Culicidae) in an urban endemic dengue area in the State of Rio de Janeiro, Brazil. Memórias do Instituto Oswaldo Cruz, 98(2), 191-198.
http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02762003000200005

97. Di Luca, M., F. Severini, L. Toma, D. Boccolini, R. Romi, M. E. Remoli, M. Sabbatucci et al. "Experimental studies of susceptibility of Italian Aedes albopictus to Zika virus." Euro Surveill. 21 (2016). http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=22468

98. Gusmão, Desiely Silva, et al. "First isolation of microorganisms from the gut diverticulum of Aedes aegypti (Diptera: Culicidae): new perspectives for an insect-bacteria association." Memórias do Instituto Oswaldo Cruz 102.8 (2007): 919-924. http://www.scielo.br/scielo.php?pid=S0074-02762007000800005&script=sci_arttext&tlng=pt

99. Apte-Deshpande, A., Paingankar, M., Gokhale, M. D., & Deobagkar, D. N. (2012). Serratia odorifera a midgut inhabitant of Aedes aegypti mosquito enhances its susceptibility to dengue-2 virus. PLoS One, 7(7), e40401. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3407224/

100. Lounibos, L. P. (2002). Invasions by insect vectors of human disease. Annual review of entomology, 47(1), 233-266. http://www.annualreviews.org/doi/abs/10.1146/annurev.ento.47.091201.145206

101. Damal, K., Murrell, E. G., Juliano, S. A., Conn, J. E., & Loew, S. S. (2013). Phylogeography of Aedes aegypti (yellow fever mosquito) in south Florida: mtDNA evidence for human-aided dispersal. The American journal of tropical medicine and hygiene, 13-0102. http://www.ajtmh.org/content/early/2013/08/01/ajtmh.13-0102.short

102. Gloria-Soria A, Brown JE, Kramer V, Hardstone Yoshimizu M, Powell JR (2014) Origin of the Dengue Fever Mosquito, Aedes aegypti, in California. PLoS Negl Trop Dis 8(7): e3029. doi:10.1371/journal.pntd.0003029

103. Brown, J. E., Scholte, E. J., Dik, M., Den Hartog, W., Beeuwkes, J., & Powell, J. R. (2011). Aedes aegypti mosquitoes imported into the Netherlands, 2010. Emerg Infect Dis, 17(12), 2335-7. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3311167/

104. Scholte, E J; Den Hartog, W; Dik, M; Schoelitsz, B; Brooks, M; Schaffner, F; Foussadier, R; Braks, M; Beeuwkes, J (2010). Introduction and control of three invasive mosquito species in the Netherlands, July-October 2010. Eurosurveillance, 15(45):19710. http://www.zora.uzh.ch/39852/1/Scholte_et_al._Eurosurveillance_15_(2010).pdf

105. Surendran, S. N., Jude, P. J., Thabothiny, V., Raveendran, S., & Ramasamy, R. (2012). Pre-imaginal development of Aedes aegypti in brackish and fresh water urban domestic wells in Sri Lanka. Journal of Vector Ecology, 37(2), 471-473. http://onlinelibrary.wiley.com/doi/10.1111/j.1948-7134.2012.00254.x/full

106. Lockwood, Jeffery A.. Six-legged Soldiers: Using Insects as Weapons of War, Oxford University Press US, 2009, p. 203, (ISBN 0195333055). https://www.amazon.com/Six-Legged-Soldiers-Using-Insects-Weapons/dp/0199733538

107. Coon, K. L., Vogel, K. J., Brown, M. R., & Strand, M. R. (2014). Mosquitoes rely on their gut microbiota for development. Molecular ecology, 23(11), 2727-2739. http://blog.caes.uga.edu/vogellab/files/2017/06/coon-et-al-2014.pdf

108. Genewatch (2017) Oxitec's Genetically Modified Mosquitoes: Failing in the field? www.genewatch.org//uploads/f03c6d66a9b354535738483c1c3d49e4/Oxitec_GWbrief_Sep17_fin.pdf

109. Coon, K. L., Valzania, L., McKinney, D. A., Vogel, K. J., Brown, M. R., & Strand, M. R. (2017). Bacteria-mediated hypoxia functions as a signal for mosquito development. Proceedings of the National Academy of Sciences, 114(27), E5362-E5369. https://pdfs.semanticscholar.org/bdbb/9eaa4274ccf65573c3043e039368728b80a5.pdf

110. John W. Norris MD (2018) Antibiotic Resistant Insects. Genetic Modification Recipe Mistake? https://view.knowledgevision.com/presentation/f685f26257334969aae998765ae6a8a9

111. Moruzzi, R. B., Barbassa, A. P., Corrêa, T. H. P., Rojas Gutierrez, L. A., & Teixeira, B. A. D. N. (2014). Aedes aegypti proliferation in stormwater retained in Best Management Practices. International Journal of Civil & Environmental Engineering, 1-6. http://www.ijens.org/Vol_14_I_02/137206-1402-8989-IJCEE-IJENS.pdf

112. Wilke, A. B., Beier, J. C., & Benelli, G. (2018). Transgenic Mosquitoes–Fact or Fiction?. Trends in parasitology. https://www.sciencedirect.com/science/article/pii/S1471492218300254

113. Irving-Bell RJ, Okoli EI, Diyelong DY, Lyimo EO, Onyia OC (1987). Septic tank mosquitoes: competition between species in central Nigeria. Medical and Veterinary Entomology, 1, 243-250. 29

114. Beserra EB, Fernandes CRM, de Sousa JT, de Freitas EM, Santos KD (2010). Efeito da qualidade da água no ciclo de vida e na atração para oviposição de Aedes aegypti (L.) (Diptera: Culicidae). Neotropical Entomology, 39, 1016-1023.

115. Burke R, Barrera R, Lewis M, Kluchinsky T, Claborn D (2010). Septic tanks as larval habitats for the mosquitoes Aedes aegypti and Culex quinquefasciatus in Playa-Playita, Puerto Rico. Medical and Veterinary Entomology, 24, 117-123.

116. Kyselkova, M., et al. (2013), Cow excrements enhance the occurrence of tetracycline resistance genes in soil regardless of their oxytetracycline content. Chemosphere. 93(10): 2413-8.

117. Ho, Y.B., et al. (2012) Simultaneous determination of veterinary antibiotics and hormone in broiler manure, soil and manure compost by liquid chromatography-tandem mass spectrometry. J Chromatogr A. 1262: 160-8.

118. Sisson TH, Hansen JM, Shah M, Hanson KE, Du M, Ling T et al. (2006) Expression of the Reverse TetracyclineTransactivator Gene Causes Emphysema-Like Changes in Mice. American Journal of Respiratory Cell and Molecular Biology, 34(5), 552 –560.

119. Ridley, E. V. (2011). The impact of chlortetracycline on Drosophila melanogaster and Aedes aegypti (PhD). University of York. Retrieved from http://etheses.whiterose.ac.uk/1642/1/Impact_of_chlortetracycline_on_Drosophila_melanogaster_and_Aede s_aegypti_EVRidley.pdf

120. Xi, Z., Ramirez, J. L., & Dimopoulos, G. (2008). The Aedes aegypti Toll Pathway Controls Dengue Virus Infection. PLOS Pathogens, 4(7), e1000098. http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1000098

121. Draft Environmental Assessment for Investigational Use of Aedes aegypti OX513A https://www.fda.gov/files/animal%20&%20veterinary/published/Oxitec-Mosquito---Draft-Environmental-Assessment.pdf

122. SDAP - Structural Database of Allergenic Proteins http://fermi.utmb.edu/SDAP/sdap_who.html

123. Ruhe, J. J., Monson, T., Bradsher, R. W., & Menon, A. (2005). Use of long-acting tetracyclines for methicillin-resistant Staphylococcus aureus infections: case series and review of the literature. Clinical infectious diseases, 40(10), 1429-1434. https://academic.oup.com/cid/article/40/10/1429/307950

124. Bandi, C., McCall, J. W., Genchi, C., Corona, S., Venco, L., & Sacchi, L. (1999). Effects of tetracycline on the filarial worms Brugia pahangi and Dirofilaria immitis and their bacterial endosymbionts Wolbachiafn1. International journal for parasitology, 29(2), 357-364. https://www.sciencedirect.com/science/article/pii/S0020751998002008

125. Zhao, Y., Schetelig, M. F., & Handler, A. M. (2020). Genetic breakdown of a Tet-off conditional lethality system for insect population control. *Nature communications*, *11*(1), 1-9. https://www.nature.com/articles/s41467-020-16807-3

126. Chadee, D. D., & Corbet, P. S. (1990). Diel patterns of oviposition indoors of the mosquito, Aedes aegypti (L.)(Diptera: Culicidae) in Trinidad, WI: a preliminary study. *Annals of Tropical Medicine & Parasitology*, *84*(1), 79-84. https://www.tandfonline.com/doi/abs/10.1080/00034983.1990.11812435

127. Syarifah, N., Rusmatini, T., Djatie, T., & Huda, F. (2008). Ovitrap ratio of Aedes aegypti larvae collected inside and outside houses in a community survey to prevent dengue outbreak, Bandung, Indonesia, 2007. *Proc Assoc Southeast Asian Nations Congr Trop Med Parasitolol*, *3*, 116-120. https://www.researchgate.net/profile/Fathul-Huda-2/publication/240642638_Ovitrap_Ratio_of_Aedes_aegypti_Larvae_collected_inside_and_outside_Houses_in_a_Community_Survey_to_Prevent_Dengue_Outbreak_Bandung_Indonesia_2007/links/53e9999d0cf2dc24b3cabf13/Ovitrap-Ratio-of-Aedes-aegypti-Larvae-collected-inside-and-outside-Houses-in-a-Community-Survey-to-Prevent-Dengue-Outbreak-Bandung-Indonesia-2007.pdf

128. Preechaporn, W., Jaroensutasinee, M., & Jaroensutasinee, K. (2006). The Larval Ecology of Aedes aegypti and Ae. albopictus in Three Topographical Areas of Southern Thailand. https://apps.who.int/iris/bitstream/handle/10665/170210/db2006v30p204.pdf

129. Garcia-Rejon, J., Loroño-Pino, M. A., Farfan-Ale, J. A., Flores-Flores, L., Rosado-Paredes, E. D. P., Rivero-Cardenas, N., ... & Eisen, L. (2008). Dengue virus–infected Aedes aegypti in the home environment. *The American journal of tropical medicine and hygiene*, *79*(6), 940-950. https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.499.9597&rep=rep1&type=pdf

130. Koyoc-Cardeña, E., Medina-Barreiro, A., Cohuo-Rodríguez, A., Pavía-Ruz, N., Lenhart, A., Ayora-Talavera, G., ... & Vazquez-Prokopec, G. (2019). Estimating absolute indoor density of Aedes aegypti using removal sampling. *Parasites & vectors*, *12*(1), 1-11. https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-019-3503-y

131. Valerio, L., Facchinelli, L., Ramsey, J. M., & Scott, T. W. (2012). Dispersal of male Aedes aegypti in a coastal village in southern Mexico. *The American journal of tropical medicine and hygiene*, *86*(4), 665. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3403765/

132. Maciel-de-Freitas, R., Eiras, Á. E., & Lourenço-de-Oliveira, R. (2006). Field evaluation of effectiveness of the BG-Sentinel, a new trap for capturing adult Aedes aegypti (Diptera: Culicidae). *Memórias do Instituto Oswaldo Cruz*, *101*, 321-325. https://www.scielo.br/j/mioc/a/CBMcbH7WRtrn7HdLYBGqLht/abstract/?lang=en&format=html

133. Kausar, M. A., Bansal, S. K., Menon, B. K., Vermani, M., & Agarwal, M. K. (2007). Mosquitoes as sources of inhalant allergens: clinicoimmunologic and biochemical studies. *Journal of Allergy and Clinical Immunology*, *120*(5), 1219. https://www.researchgate.net/publication/6122752_Mosquitoes_as_sources_of_inhalant_allergens_Clinicoimmunologic_and_biochemical_studies

134. Cantillo, J. F., Puerta, L., Lafosse-Marin, S., Subiza, J. L., Caraballo, L., & Fernandez-Caldas, E. (2017). Allergens involved in the cross-reactivity of Aedes aegypti with other arthropods. *Annals of Allergy, Asthma & Immunology*, *118*(6), 710-718. https://www.sciencedirect.com/science/article/abs/pii/S1081120617302090

135. GUPTA, S., Jain, S., CHAUDHRY, S., & Agarwal, M. K. (1990). Role of insects as inhalant allergens in bronchial asthma with special reference to the clinical characteristics of patients. *Clinical & Experimental Allergy*, *20*(5), 519-524. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2222.1990.tb03144.x

136. Coattrenec, Y., Jaques, D., Jandus, P., Harr, T., & Spoerl, D. (2017). Anaphylactic shock following castor bean contact: a case report. *Allergy, Asthma & Clinical Immunology*, *13*(1), 1-3. https://aacijournal.biomedcentral.com/articles/10.1186/s13223-017-0221-x

137. Asam, C., Batista, A.L., Moraes, A.H., de Paula, V.S., Almeida, F.C.L., Aglas, L., Kitzmüller, C., Bohle, B., Ebner, C., Ferreira, F. and Wallner, M., 2014. Bet v 1–a Trojan horse for small ligands boosting allergic sensitization?. *Clinical & Experimental Allergy*, *44*(8), pp.1083-1093. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6910946/

138. Shaw WR, Holmdahl IE, Itoe MA, Werling K, Marquette M, Paton DG, et al. (2020) Multiple blood feeding in mosquitoes shortens the *Plasmodium falciparum* incubation period and increases malaria transmission potential. PLoS Pathog 16(12): e1009131. https://doi.org/10.1371/journal.ppat.1009131

139. Campos, S.S., Fernandes, R.S., Dos Santos, A.A.C., de Miranda, R.M., Telleria, E.L., Ferreira-de-Brito, A., de Castro, M.G., Failloux, A.B., Bonaldo, M.C. and Lourenço-de-Oliveira, R., 2017. Zika virus can be venereally transmitted between Aedes aegypti mosquitoes. *Parasites & vectors*, *10*(1), pp.1-4. https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-017-2543-4

140. Pereira-Silva, J.W., Nascimento, V.A.D., Belchior, H.C.M., Almeida, J.F., Pessoa, F.A.C., Naveca, F.G. and Ríos-Velásquez, C.M., 2017. First evidence of Zika virus venereal transmission in Aedes aegypti mosquitoes. *Memórias do Instituto Oswaldo Cruz*, *113*, pp.56-61. https://www.scielo.br/j/mioc/a/4sgVDfrRgbjYVshNPW9hL6r/?lang=en

141. Calle-Tobón, A., Pérez-Pérez, J., Rojo, R., Rojas-Montoya, W., Triana-Chavez, O., Rúa-Uribe, G. and Gómez-Palacio, A., 2020. Surveillance of Zika virus in field-caught Aedes aegypti and Aedes albopictus suggests important role of male mosquitoes in viral populations maintenance in Medellín, Colombia. *Infection, Genetics and Evolution*, *85*, p.104434. https://www.sciencedirect.com/science/article/abs/pii/S1567134820302653

142.Helinski, M.E. and Harrington, L.C., 2011. Male mating history and body size influence female fecundity and longevity of the dengue vector Aedes aegypti. *Journal of medical entomology*, *48*(2), pp.202-211. https://academic.oup.com/jme/article-abstract/48/2/202/892019

143. Mosquito Protection: What You Need to Know https://fla-keys.com/mosquito-protection/

144. Dutra, H.L.C., Rocha, M.N., Dias, F.B.S., Mansur, S.B., Caragata, E.P. and Moreira, L.A., 2016. Wolbachia blocks currently circulating Zika virus isolates in Brazilian Aedes aegypti mosquitoes. *Cell host & microbe*, *19*(6), pp.771-774. https://www.sciencedirect.com/science/article/pii/S1931312816301573

145. Van den Hurk, A.F., Hall-Mendelin, S., Pyke, A.T., Frentiu, F.D., McElroy, K., Day, A., Higgs, S. and O'Neill, S.L., 2012. Impact of Wolbachia on infection with chikungunya and yellow fever viruses in the mosquito vector Aedes aegypti. *PLoS neglected tropical diseases*, *6*(11), p.e1892. https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0001892

146. Mains, J.W., Kelly, P.H., Dobson, K.L., Petrie, W.D. and Dobson, S.L., 2019. Localized control of Aedes aegypti (Diptera: Culicidae) in Miami, FL, via inundative releases of Wolbachia-infected male mosquitoes. *Journal of medical entomology*, *56*(5), pp.1296-1303. https://academic.oup.com/jme/article-abstract/56/5/1296/5475244

147. Ryan, P.A., Turley, A.P., Wilson, G., Hurst, T.P., Retzki, K., Brown-Kenyon, J., Hodgson, L., Kenny, N., Cook, H., Montgomery, B.L. and Paton, C.J., 2019. Establishment of wMel Wolbachia in Aedes aegypti mosquitoes and reduction of local dengue transmission in Cairns and surrounding locations in northern Queensland, Australia. *Gates open research*, *3*. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6801363/

148. Gandin Filho, R., 2019. Flutuação da dengue em uma cidade de médio porte, com solturas de mosquitos geneticamente modificados: um estudo longitudinal. http://repositorio.unicamp.br/jspui/handle/REPOSIP/333848

149. Genetically modified mosquitoes' survival rate concealed https://foe.org/news/2012-01-genetically-modified-mosquitoes-survival-rate/

150. Ciota, A.T., Bialosuknia, S.M., Ehrbar, D.J. and Kramer, L.D., 2017. Vertical transmission of Zika virus by Aedes aegypti and Ae. albopictus mosquitoes. *Emerging infectious diseases*, *23*(5), p.880. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5403030/

151. Villarreal, S.M., Pitcher, S., Helinski, M.E., Johnson, L., Wolfner, M.F. and Harrington, L.C., 2018. Male contributions during mating increase female survival in the disease vector mosquito Aedes aegypti. *Journal of insect physiology*, *108*, pp.1-9. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5988987/

152. Evans, B.R., Kotsakiozi, P., Costa-da-Silva, A.L., Ioshino, R.S., Garziera, L., Pedrosa, M.C., Malavasi, A., Virginio, J.F., Capurro, M.L. and Powell, J.R., 2019. Transgenic Aedes aegypti mosquitoes transfer genes into a natural population. *Scientific reports*, *9*(1), pp.1-6. https://www.nature.com/articles/s41598-019-49660-6?fbclid=IwAR0dIwaxuWIbkxNEfPWgnTSFFF_tkrqlS25HN88GGKC41n6cWEB2Wb3_WnQ

153. Ponlawat, A. and Harrington, L.C., 2009. Factors associated with male mating success of the dengue vector mosquito, Aedes aegypti. *The American journal of tropical medicine and hygiene*, *80*(3), pp.395-400. https://www.ajtmh.org/view/journals/tpmd/80/3/article-p395.xml?tab_body=abstract

154. Carvalho, D.O., Chuffi, S., Ioshino, R.S., Marques, I.C., Fini, R., Costa, M.K., Araújo, H.R., Costa-da-Silva, A.L., Kojin, B.B. and Capurro, M.L., 2018. Mosquito pornoscopy: Observation and interruption of Aedes aegypti copulation to determine female polyandric event and mixed progeny. *Plos one*, *13*(3), p.e0193164. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0193164

155. Orlando Sentinel (2021) Key West has a chicken problem, and city is trying to do something about it. https://www.orlandosentinel.com/news/florida/os-ne-key-west-chickens-20210125-g2tj2c4e65dy3b2m7uoxq47owm-story.html

156. Bargielowski, I., Kaufmann, C., Alphey, L., Reiter, P. and Koella, J., 2012. Flight performance and teneral energy reserves of two genetically-modified and one wild-type strain of the yellow fever mosquito Aedes aegypti. *Vector-Borne and Zoonotic Diseases*, *12*(12), pp.1053-1058. https://www.researchgate.net/profile/Jacob-Koella/publication/230572049_Flight_Performance_and_Teneral_Energy_Reserves_of_Two_Genetically-Modified_and_One_Wild-Type_Strain_of_the_Yellow_Fever_Mosquito_Aedes_aegypti/links/5632119508ae0530378e6a04/Flight-Performance-and-Teneral-Energy-Reserves-of-Two-Genetically-Modified-and-One-Wild-Type-Strain-of-the-Yellow-Fever-Mosquito-Aedes-aegypti.pdf

157. Pascini, T.V., Ramalho-Ortigão, M., Ribeiro, J.M., Jacobs-Lorena, M. and Martins, G.F., 2020. Transcriptional profiling and physiological roles of Aedes aegypti spermathecal-related genes. *BMC genomics*, *21*(1), pp.1-18. https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-020-6543-y

158. Sebastian, A., Sein, M.M., Thu, M.M. and Corbet, P.S., 1990. Suppression of Aedes aegypti (Diptera: Culicidae) using augmentative release of dragonfly larvae (Odonata: Libellulidae) with community participation in Yangon, Myanmar1. *Bulletin of Entomological Research*, *80*(2), pp.223-232. https://www.cambridge.org/core/journals/bulletin-of-entomological-research/article/abs/suppression-of-aedes-aegypti-diptera-culicidae-using-augmentative-release-of-dragonfly-larvae-odonata-libellulidae-with-community-participation-in-yangon-myanmar1/3AD30472BFAF5F0CA448C7C11B7608F0

159. Samanmali, C., Udayanga, L., Ranathunge, T., Perera, S.J., Hapugoda, M. and Weliwitiya, C., 2018. Larvicidal potential of five selected dragonfly nymphs in Sri Lanka over Aedes aegypti (Linnaeus) larvae under laboratory settings. *BioMed research international*, *2018*. https://www.hindawi.com/journals/bmri/2018/8759459/

160. Leite Alves, A.C., Iarley da Silva, T., Roberto de Azevedo, F., Rocha Virgulino, R., Trummer Leandro Costa, C.E., Valmir Feitosa, J., Pereira Lima, E. and Azevedo, R., 2018. Actividad atractiva de extractos vegetales para la oviposición de Aedes aegypti L.(Diptera: Culicidae). *Idesia (Arica)*, *36*(2), pp.225-231. https://scielo.conicyt.cl/scielo.php?pid=S0718-34292018000200225&script=sci_arttext&tlng=e

161. IBÁÑEZ-BERNAL, S.E.R.G.I.O., Briseno, B., Mutebi, J.P., Argot, E., Rodriguez, G., MARTINEZ-CAMPOS, C.A.R.M.E.N., Paz, R., ROMÁN, P.D.L.F.S., TAPIA-CONYER, R.O.B.E.R.T.O. and Flisser, A., 1997. First record in America of Aedes albopictus naturally infected with dengue virus during the 1995 outbreak at

Reynosa, Mexico. *Medical and veterinary entomology*, *11*(4), pp.305-309. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2915.1997.tb00413.x

162. Holick, J., Kyle, A., Ferraro, W., Delaney, R.R. and Iwaseczko, M., 2002. Discovery of Aedes albopictus infected with West Nile virus in southeastern Pennsylvania. *Journal of the American Mosquito Control Association*, *18*(2), pp.131-131. https://europepmc.org/article/med/12083357

163. Moore, C.G., 1999. Aedes albopictus in the United States: current status and prospects for further spread. *Journal of the American Mosquito Control Association*, *15*(2), pp.221-227. https://www.biodiversitylibrary.org/content/part/JAMCA/JAMCA_V15_N2_P221-227.pdf

164. Gerhardt, R.R., Gottfried, K.L., Apperson, C.S., Davis, B.S., Erwin, P.C., Smith, A.B., Panella, N.A., Powell, E.E. and Nasci, R.S., 2001. First isolation of La Crosse virus from naturally infected Aedes albopictus. Emerging infectious diseases, 7(5), p.807. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2631884/

165. Akhtar, Z.R., Tariq, K., Mavian, C., Ali, A., Ullah, F., Zang, L.S., Ali, F., Nazir, T. and Ali, S., 2021. Trophic transfer and toxicity of heavy metals from dengue mosquito Aedes aegypti to predator dragonfly Tramea cophysa. *Ecotoxicology*, pp.1-8. https://link.springer.com/article/10.1007/s10646-021-02448-9

166. Amos, B.A., Hoffmann, A.A., Staunton, K.M., Lau, M.J., Burkot, T.R. and Ross, P.A., 2021. Strong attraction of male mosquitoes (Aedes aegypti) to humans. *bioRxiv*. https://www.researchgate.net/publication/351860499_Strong_attraction_of_male_mosquitoes_Aedes_aegypti_to_humans

167. Kramer, I.M., Kreß, A., Klingelhöfer, D., Scherer, C., Phuyal, P., Kuch, U., Ahrens, B., Groneberg, D.A., Dhimal, M. and Müller, R., 2020. Does winter cold really limit the dengue vector Aedes aegypti in Europe?. *Parasites & vectors*, *13*(1), pp.1-13. https://pure.itg.be/ws/portalfiles/portal/12753582/2020pvec0178.pdf

168. Cantillo, J.F., Puerta, L., Puchalska, P., Lafosse-Marin, S., Subiza, J.L. and Fernández-Caldas, E., 2017. Allergenome characterization of the mosquito Aedes aegypti. *Allergy*, *72*(10), pp.1499-1509. https://www.researchgate.net/profile/Jose-Cantillo/publication/314017873_Allergenome_characterization_of_the_mosquito_Aedes_aegypti/links/5d24caae458515c11c217020/Allergenome-characterization-of-the-mosquito-Aedes-aegypti.pdf

169. Ramasamy, R., Surendran, S.N., Jude, P.J., Dharshini, S. and Vinobaba, M., 2011. Larval development of Aedes aegypti and Aedes albopictus in peri-urban brackish water and its implications for transmission of arboviral diseases. *PLoS neglected tropical diseases*, *5*(11), p.e1369. https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0001369

170. Knudsen, K.E., Reid, W.R., Barbour, T.M., Bowes, L.M., Duncan, J., Philpott, E., Potter, S. and Scott, M.J., 2020. Genetic variation and potential for resistance development to the tTA overexpression lethal system in insects. *G3: Genes, Genomes, Genetics*, *10*(4), pp.1271-1281. https://academic.oup.com/g3journal/article/10/4/1271/6026188?login=true

171. de Moura Rodrigues, M., Marques, G.R.A.M., Serpa, L.L.N., de Brito Arduino, M., Voltolini, J.C., Barbosa, G.L., Andrade, V.R. and de Lima, V.L.C., 2015. Density of Aedes aegypti and Aedes albopictus and its association with number of residents and meteorological variables in the home environment of

dengue endemic area, São Paulo, Brazil. *Parasites & vectors*, *8*(1), pp.1-9. https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-015-0703-y

172. Massanyi, P., Stawarz, R., Halo, M., Formicki, G., Lukac, N., Cupka, P., Schwarcz, P., Kovacik, A., Tusimova, E. and Kovacik, J., 2014. Blood concentration of copper, cadmium, zinc and lead in horses and its relation to hematological and biochemical parameters. *Journal of Environmental Science and Health, Part A*, *49*(8), pp.973-979. https://www.tandfonline.com/doi/abs/10.1080/10934529.2014.894322

173. Janiszewska, J. and Cieśla, A., 2002. Concentration of cadmium and lead in horse blood serum and hair in relation to season and environment. *Electronic Journal of Polish Agricultural Universities*, *5*(1). www.ejpau.media.pl/articles/volume5/issue1/animal/art-06.pdf

174. Gloria-Soria, A., Lima, A., Lovin, D.D., Cunningham, J.M., Severson, D.W. and Powell, J.R., 2018. Origin of a high-latitude population of Aedes aegypti in Washington, DC. The American journal of tropical medicine and hygiene, 98(2), p.445. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5929211/

175. da Silva Paixão, K., de Castro Pereira, I., Lopes Alves Bottini, L. and Eduardo Eiras, Á., 2015. Volatile semiochemical-conditioned attraction of the male yellow fever mosquito, Aedes aegypti, to human hosts. *Journal of Vector Ecology*, *40*(1), pp.1-6. https://onlinelibrary.wiley.com/doi/full/10.1111/jvec.12143

176. Tallon, A.K., Hill, S.R. and Ignell, R., 2019. Sex and age modulate antennal chemosensory-related genes linked to the onset of host seeking in the yellow-fever mosquito, Aedes aegypti. *Scientific reports*, *9*(1), pp.1-13. https://www.nature.com/articles/s41598-018-36550-6

177. Nelson, M.J., Self, L.S., Pant, C.P. and Usman, S., 1978. Diurnal periodicity of attraction to human bait of Aedes aegypti (Diptera: Culicidae) in Jakarta, Indonesia. *Journal of medical entomology*, *14*(5), pp.504-510. https://academic.oup.com/jme/article-abstract/14/5/504/2219453

178. Hartberg, W.K., 1971. Observations on the mating behaviour of Aedes aegypti in nature. *Bulletin of the World Health Organization*, *45*(6), p.847. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2428001/?page=4

179. Cabrera, M. and Jaffe, K., 2007. An aggregation pheromone modulates lekking behavior in the vector mosquito Aedes aegypti (Diptera: Culicidae). *Journal of the American Mosquito Control Association*, *23*(1), pp.1-10. https://pubmed.ncbi.nlm.nih.gov/17536361/

180. Fawaz, E.Y., Allan, S.A., Bernier, U.R., Obenauer, P.J. and Diclaro, J.W., 2014. Swarming mechanisms in the yellow fever mosquito: aggregation pheromones are involved in the mating behavior of Aedes aegypti. *Journal of Vector Ecology*, *39*(2), pp.347-354. https://onlinelibrary.wiley.com/doi/full/10.1111/jvec.12110

181. Mercer, D.K., Scott, K.P., Melville, C.M., Glover, L.A. and Flint, H.J., 2001. Transformation of an oral bacterium via chromosomal integration of free DNA in the presence of human saliva. *FEMS Microbiology Letters*, *200*(2), pp.163-167. https://academic.oup.com/femsle/article/200/2/163/597029?login=true

182. Aedes aegypti and Aedes albopictus Mosquitoes in California by County/City https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/AedesDistributionMap.pdf

183. McCracken, M.K., Christofferson, R.C., Grasperge, B.J., Calvo, E., Chisenhall, D.M. and Mores, C.N., 2014. Aedes aegypti salivary protein "aegyptin" co-inoculation modulates dengue virus infection in the vertebrate host. *Virology*, *468*, pp.133-139. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4254699/

184. Sri-In, C., Weng, S.C., Chen, W.Y., Wu-Hsieh, B.A., Tu, W.C. and Shiao, S.H., 2019. A salivary protein of Aedes aegypti promotes dengue-2 virus replication and transmission. *Insect biochemistry and molecular biology*, *111*, p.103181. https://www.sciencedirect.com/science/article/abs/pii/S0965174819301237

185. Bowatte, G., Perera, P., Senevirathne, G., Meegaskumbura, S. and Meegaskumbura, M., 2013. Tadpoles as dengue mosquito (Aedes aegypti) egg predators. Biological Control, 67(3), pp.469-474. https://www.researchgate.net/profile/Madhava-Meegaskumbura/publication/258104790_Tadpoles_as_Dengue_Mosquito_Aedes_aegypti_egg_predators/links/5a56e04b0f7e9bf2a536d940/Tadpoles-as-Dengue-Mosquito-Aedes-aegypti-egg-predators.pdf

186. Ritchie, S. A., & SA, R. (1982). The green tree frog (Hyla cinerea) as a predator of mosquitoes in Florida. https://www.biodiversitylibrary.org/content/part/JAMCA/MN_V42_N4_P619.pdf

187. Hu, Y.C., Tang, Y., Chen, Z.Q., Chen, J.Y. and Ding, G.H., 2019. Evaluation of the sensitivity of Microhyla fissipes tadpoles to aqueous cadmium. *Ecotoxicology*, *28*(10), pp.1150-1159. https://link.springer.com/article/10.1007/s10646-019-02117-y

188. DuRant, S.E. and Hopkins, W.A., 2008. Amphibian predation on larval mosquitoes. *Canadian journal of Zoology*, *86*(10), pp.1159-1164. https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.942.4932&rep=rep1&type=pdf

189. Brodman, R. and Dorton, R., 2006. The effectiveness of pond-breeding salamanders as agents of larval mosquito control. *Journal of Freshwater Ecology*, *21*(3), pp.467-474. https://www.tandfonline.com/doi/pdf/10.1080/02705060.2006.9665024

190. Federal Register Volume 59, Number 219 (Tuesday, November 15, 1994

https://www.govinfo.gov/content/pkg/FR-1994-11-15/html/94-28029.htm

191. Watters, A.M., Rowland, F.E. and Semlitsch, R.D., 2018. Larval salamanders are as effective at short-term mosquito predation as mosquitofish. *Canadian Journal of Zoology*, *96*(10), pp.1165-1169. https://tspace.library.utoronto.ca/bitstream/1807/90475/1/cjz-2017-0267.pdf

192. Naz, S., Akbar, H., Habib, S., Rais, M.F. and Malik, M.A., 2019. Role of anuran tadpoles as biological control for dengue larvae. *International Journal of Mosquito Research*, *6*, pp.22-26. https://www.researchgate.net/profile/Saira-Naz-2/publication/338540201_Role_of_anuran_tadpoles_as_biological_control_for_dengue_larvae/links/5e1aa01ea6fdcc28376ba774/Role-of-anuran-tadpoles-as-biological-control-for-dengue-larvae.pdf

193. Oxitec Expands Friendly™ Aedes Technology in Brazil in Support of City Dengue Control Program as Dengue Season Begins 24 November 2020 https://www.oxitec.com/en/news/oxitec-expands-friendly-aedes-technology-in-brazil-in-support-of-city-dengue-control-program-as-dengue-season-begins

194. Pinguicula primuliflora Wood & Godfrey https://georgiabiodiversity.org/natels/profile?es_id=15432

195. Kenda Robertson (2007) Our little friends
https://www.orlandosentinel.com/news/os-xpm-2007-09-09-anoles09-story.html

196. United States Department of Agriculture. Velvety Free-Tailed Bat
https://www.fs.usda.gov/detail/elyunque/learning/nature-science/?cid=fsbdev3_042947

197. Zhou, J., Xiao, K., Wei, B., Wang, Z., Tian, Y., Tian, Y. and Song, Q., 2014. Bioaccumulation of Cry1Ab protein from an herbivore reduces anti-oxidant enzyme activities in two spider species. *PloS one*, *9*(1), p.e84724. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0084724

198. Paula, D.P. and Andow, D.A., 2016. Uptake and bioaccumulation of Cry toxins by an aphidophagous predator. *Environmental Pollution*, *209*, pp.164-168.
https://www.sciencedirect.com/science/article/abs/pii/S0269749115301962

199. Bundschuh, R., Bundschuh, M., Otto, M. and Schulz, R., 2019. Food-related exposure to systemic pesticides and pesticides from transgenic plants: evaluation of aquatic test strategies. *Environmental Sciences Europe*, *31*(1), pp.1-13. https://link.springer.com/article/10.1186/s12302-019-0266-1

200. Lin, Z., Li, J., Luan, Y. and Dai, W., 2020. Application of algae for heavy metal adsorption: A 20-year meta-analysis. *Ecotoxicology and environmental safety*, *190*, p.110089.
https://www.sciencedirect.com/science/article/abs/pii/S0147651319314204

201. Waldeck, W., Heidenreich, E., Mueller, G., Wiessler, M., Tóth, K. and Braun, K., 2012. ROS-mediated killing efficiency with visible light of bacteria carrying different red fluorochrome proteins. Journal of Photochemistry and Photobiology B: Biology, 109, pp.28-33.
https://www.sciencedirect.com/science/article/abs/pii/S1011134412000139

202. Zakrzewska, K.E., Samluk, A., Pluta, K.D. and Pijanowska, D.G., 2014. Evaluation of the effects of antibiotics on cytotoxicity of EGFP and DsRed2 fluorescent proteins used for stable cell labeling. *Acta Biochimica Polonica*, *61*(4). https://ojs.ptbioch.edu.pl/index.php/abp/article/view/1850